ANNUAL REVIEWS Further
Quick links to online content

Ann. Rev. Pharmacol. Toxicol. 1977. 17:441–77
Copyright © 1977 by Annual Reviews Inc. All rights reserved

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org
by Central College on 12/14/11. For personal use only.

SELECTIVE CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITORS AS POTENTIAL THERAPEUTIC AGENTS

❖6690

Benjamin Weiss and William N. Hait
Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

INTRODUCTION

Not thirty years have passed since Earl Sutherland, Ted Rall, and their co-workers first suggested that a heat-stable cyclic nucleotide mediates the actions of certain hormones and neurotransmitters. This original handful of cyclic nucleotide enthusiasts has now swelled to hundreds and encompasses biologists of every discipline. As one might expect, the early studies concentrated largely on the biochemical and physiological actions of cyclic nucleotides and on the enzymes responsible for their biosynthesis (nucleotide cyclases) and hydrolysis (cyclic nucleotide phosphodiesterases); relatively little was done to manipulate the intracellular concentrations of cyclic nucleotides by pharmacological means. Now, however, in the face of mounting evidence that cyclic nucleotides may be involved in the etiology or pathogenesis of certain diseases, increasing numbers of investigators have turned their attention to finding pharmacologic agents that will selectively alter the intracellular concentration or the action of cyclic nucleotides. These agents fall into three general categories: compounds that alter the activity of the nucleotide cyclases, the cyclic nucleotide phosphodiesterases, or the cyclic nucleotide–dependent protein kinases.

The evidence that the cyclic nucleotide phosphodiesterases exist in several molecular forms and that these isozymes are unequally distributed in tissue makes the phosphodiesterases particularly suitable targets for pharmacologic manipulation, for it suggests that by finding selective inhibitors of the different phosphodiesterase isozymes, one may be able to raise the concentration of cyclic nucleotides in discrete cell types. Through the selective inhibition of the major phosphodiesterase isozyme of a diseased tissue, it may then be possible to alter the course of diseases characterized by an abnormal metabolism of cyclic nucleotides.

The present paper reviews the findings that suggest that cyclic nucleotides may be involved in certain diseases and summarizes the experimental results that support the notion that certain inhibitors of cyclic nucleotide phosphodiesterase might serve as effective and specific therapeutic agents.

ROLE OF CYCLIC NUCLEOTIDES IN BIOLOGY

The cyclic nucleotides, adenosine cyclic 3',5'-monophosphate (cyclic AMP), and guanosine cyclic 3',5'-monophosphate (cyclic GMP) have been shown to regulate innumerable biological processes [for reviews see (1–15)]. Cyclic AMP not only mediates the actions of most biogenic amines and polypeptide hormones (16, 17), but also appears to influence such fundamental processes as cell division (18), fertilization (19), embryonic growth and differentiation (20), blood production (21–23), smooth muscle tone (24, 25), cardiac contractility and metabolism (26–28), the function of the central nervous system (29) and autonomic nervous system (30), vision (31), immunological responses (32, 33), gonadal function (34), and the release of stored intracellular materials such as insulin (35–37), histamine (38, 39), and lysosomal enzymes (40).

Several of the actions of cyclic GMP appear to be opposite to those of cyclic AMP. These observations have prompted the so-called yin-yang hypothesis (41).

Since the cyclic nucleotides influence the most basic processes in biology, any abnormality in the intracellular concentration of these compounds might disrupt normal physiological function and lead to disease. The following sections explore this possibility and suggest certain approaches by which one may use drugs to restore the proper balance in the cyclic nucleotide system.

ROLE OF CYCLIC NUCLEOTIDES IN DISEASE

As mentioned earlier, there are three major sites at which the cyclic nucleotide system may be altered in diseases and, accordingly, three general sites at which drugs may interact to modulate disease processes. One site is on the cyclic nucleotide cyclases which catalyze the synthesis of cyclic nucleotides from the high energy phosphate compounds, adenosine triphosphate and guanosine triphosphate; another is at the level of the cyclic nucleotide phosphodiesterases, a group of hydrolytic enzymes, which exist in several molecular forms and catalyze the degradation of cyclic nucleotides; and a third site is on the cyclic nucleotide–dependent protein kinases which, when activated by the cyclic nucleotides, cause the phosphorylation of other intracellular proteins leading ultimately to a biological response.

Diseases Associated with Defects in the Cyclic Nucleotide System

Table 1 outlines the diseases which are associated with abnormalities in each of the different steps of cyclic nucleotide physiology. An example of a disease which is associated with an abnormality in adenylate cyclase is nephrogenic diabetes insipidus. In this disorder, which is characterized by an inability to concentrate urine in response to antidiuretic hormone (ADH), the adenylate cyclase receptor in the

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org
by Central College on 12/14/11. For personal use only.

Table 1 Diseases associated with abnormalities in the concentration or metabolism of cyclic nucleotides

Disease	Tissue	Cyclic nucleotide concentration			Enzyme activity					References
		cyclic AMP		cyclic GMP	Phosphodiesterase		Adenylate cyclase		Guanylate cyclase	Protein kinase	
		basal	stim.[a]		cyclic AMP	cyclic GMP	basal	stim.[b]			
ASTHMA	leucocytes								decrease		265
	leucocytes		decrease								213
	leucocytes							decrease			266
	leucocytes		decrease								267
	urine		decrease								268
CANCER	adrenal cortex				decrease	decrease					269
	adrenal cortex		decrease								47
	glioblastoma						increase				280
	hepatoma	increase		increase							270
	hepatoma						decrease	decrease			271
	hepatoma	decrease		increase							65
	hepatoma	increase			decrease		increase				272
	hepatoma	increase									273
	hepatoma									decrease	66
	hepatoma				c						274
	hepatoma						decrease				275
	hepatoma				c						276
	hepatoma	decrease									183
	hepatoma								d		277
	thymus						increase	decrease			50,278
	thymus	decrease			increase						50
	lymphocytes		decrease								184

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Table 1 *(Continued)*

Disease	Tissue	Cyclic nucleotide concentration: cyclic AMP basal	cyclic AMP stim.[a]	cyclic GMP	Enzyme activity: Phosphodiesterase cyclic AMP	Phosphodiesterase cyclic GMP	Adenylate cyclase basal	Adenylate cyclase stim.[b]	Guanylate cyclase	Protein kinase	References
	lymphocytes	decrease									264
	leukocytes							decrease			49
	lymphocytes				increase	increase					60,61
	spleen				decrease						279
	mammary	decrease									281
	mammary				increase	increase					156
	mammary	increase			increase						282
	mammary						increase				283
	mammary	increase									284
	plant				increase						285
	skin	increase	decrease								286
	skin melanoma						decrease				287
	skin	decrease									288
	skin	increase			increase	increase					289
	thyroid							decrease			48
	transformed cells						decrease				45
	transformed cells						decrease				290
	transformed cells						decrease				291
	transformed cells						decrease				44

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Table 1 *(Continued)*

	transformed cells	decrease							292
	transformed cells				decrease				293
	transformed cells		decrease		decrease			decrease	51
	transformed cells					decrease	decrease		275
	transformed cells			increase		increase			181
	transformed cells					increase			294
	transformed cells					decrease			294
CARDIOVASCULAR									
Atherosclerosis		increase							295
Congestive failure	cardiac						decrease		242--245, 296
	cardiac					decrease			243
Hypertension	amniotic fluid	increase							297
	aorta					increase			298
	aorta	decrease		decrease		decrease			299
	aorta	decrease		increase			decrease		52
	cardiac	decrease		decrease		decrease			300
	plasma	increase							86
	urine	increase							301
Myocardial infarction	plasma	increase							85

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Table 1 *(Continued)*

Disease	Tissue	Cyclic nucleotide concentration: cyclic AMP basal	Cyclic nucleotide concentration: cyclic AMP stim.[a]	Cyclic nucleotide concentration: cyclic GMP	Enzyme activity: Phosphodiesterase cyclic AMP	Enzyme activity: Phosphodiesterase cyclic GMP	Enzyme activity: Adenylate cyclase basal	Enzyme activity: Adenylate cyclase stim.[b]	Enzyme activity: Guanylate cyclase	Enzyme activity: Protein kinase	References
DOWN'S SYNDROME	saliva	increase									88
ENDOCRIN-OPATHIES											
Diabetes insipidus	kidney							decrease			43
	urine		decrease					decrease			42
	urine		decrease								77
Diabetes mellitus	adipocytes				decrease						54
	heart				decrease						56
	liver	increase									56
	pancreas				decrease						54
	plasma				decrease						55
	saliva	decrease									87
Hyperpara-thyroidism	urine	increase									73-75, 302,303
Hypopara-thyroidism	urine	decrease									73-75, 302,303
Hyperthyroid-ism	urine	increase									71,304
	urine		increase								72

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Table 1 *(Continued)*

Hypothyroidism	adipocytes		decrease						305
Pseudohypoparathyroidism	urine		decrease						75
EYE DISEASE	retina				decrease	decrease			57,58
	retina			increase					306
NEUROPSYCHIATRIC DISEASES									
CNS damage	CSF	increase							307
Depression	CSF	decrease							80
	urine	decrease							79-81
Epilepsy	cerebrum		decrease						309
	CSF	increase							307
	forebrain	increase							308
Mania	CSF	increase							80
	urine	increase							79-81
OBESITY	adipose tissue		decrease		increase		increase		53
	adipose tissue							decrease	232
PSORIASIS	epidermis	decrease							204
	epidermis	decrease		increase					205
	epidermis						decrease	decrease	207
	epidermis				increase				208

[a] Hormonally stimulated increase in cyclic AMP concentration.

[b] Hormonally stimulated increase in adenylate cyclase activity.

[c] Increase in low K_m phosphodiesterase and decrease in high K_m phosphodiesterase.

[d] Increase in particulate guanylate cyclase and decrease in soluble guanylate cyclase.

renal tubule is insensitive to ADH (42, 43). Similarly, several forms of cancer, which have abnormally low intracellular concentrations of cyclic AMP, have a reduction in adenylate cyclase activity or a reduction in its hormonal responsiveness (44–50). Other forms of cancer reportedly have a reduced activity of guanylate cyclase as well (51).

There is also evidence that tissues of animals with certain diseases have an abnormal activity of cyclic AMP phosphodiesterase. These include aortas of spontaneously hypertensive rats (52), adipocytes of congenitally obese mice (53), pancreas and adipose tissue of spontaneously diabetic mice (54), plasma (55) and hearts (56) of diabetic rats, and retina of mice with an inherited retinal degenerative disease (57, 58). Our studies have shown that the activities of both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase are increased tenfold in leukemic lymphocytes compared with that of normal lymphocytes (59–61).

Certain diseases may also have a defect in the major site at which cyclic nucleotides act, i.e. at the protein kinases. For example, in a variant of pseudohypoparathyroidism (Type II), although cyclic AMP is produced in response to parathyroid hormone, the cyclic nucleotide fails to induce the normal physiological response (62). A similar effect is seen in certain murine lymphomas. Whereas in most lymphomas cyclic AMP is cytotoxic, in a mutant cell line lacking a specific cyclic AMP–dependent protein kinase, cyclic AMP fails to exert a carcinostatic effect (63, 64). Abnormalities in the intracellular binding of cyclic nucleotides have also been seen in other forms of cancer (65–67) and in diabetes (68).

Measurement of Cyclic Nucleotides in Tissue and Extracellular Fluid As a Diagnostic Aid

The findings that certain diseases are associated with an abnormal metabolism of cyclic nucleotides suggests that one might find a different concentration of cyclic nucleotides in the diseased tissue or in one of the extracellular fluid compartments (e.g. plasma, urine, or cerebrospinal fluid) of diseased individuals. This proved to be the case (for review see ref. 69). For example, in studies of the urinary excretion of cyclic nucleotides it was reported that the concentration of cyclic AMP in urine is increased in patients with diabetes (70), hyperthyroidism (71, 72), and hyperparathyroidism (73, 74). Moreover, injection of parathyroid hormone causes a large increase in urinary cyclic AMP in patients with hypoparathyroidism but not in patients with pseudohypoparathyroidism (75, 76). Urinary cyclic AMP does not increase in response to ADH in patients with nephrogenic diabetes insipidus (42) but does increase in response to ADH in patients who are ADH deficient (77). The reports of urinary excretion of cyclic AMP in manic patients are conflicting. Some showed an increase in cyclic AMP excretion in mania (79–81), whereas more recent studies failed to find such differences (82, 83). Finally, it has been found that the concentration of cyclic GMP in urine of rats bearing hepatomas is increased in proportion to the rate of tumor growth (84).

In studies of the concentration of cyclic nucleotides in plasma of diseased individuals, it was found that cyclic AMP increased following massive myocardial infarction (85) and increased in patients with hypertension (86). In cerebrospinal fluid,

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

cyclic AMP was reported to be elevated during the manic phase and decreased during the depressive phase of manic depressive disease (80). In gingival fluid, cyclic AMP was decreased in diabetic patients (87). In saliva, cyclic AMP was increased in children with Down's syndrome (88).

With the exception of the case of hypoparathyroidism, none of the above studies has as yet resulted in the development of specific diagnostic tests. Nevertheless, the potential of finding an early diagnostic test based on a correlation between the concentration of cyclic nucleotide in extracellular fluids with a particular disease state still exists, and this area of study should continue to be explored.

These studies point to the possibility that cyclic nucleotides are important factors in the etiology or pathogenesis of many diseases and suggest that by restoring the imbalance in the intracellular concentration of cyclic nucleotides, one may eliminate the cause or alleviate the symptoms of the disease. The enormous problem presented to the pharmacologist is not only how to alter the intracellular concentration of cyclic nucleotides, but more importantly, how to alter the concentration of each of the cyclic nucleotides selectively in discrete cell types.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

DEVELOPMENT OF THERAPEUTIC AGENTS THAT ALTER CYCLIC NUCLEOTIDE METABOLISM

General Considerations

A major problem in using drugs affecting cyclic nucleotide metabolism to alter physiological responses is the ubiquitous nature of the cyclic nucleotides and the wide variety of responses they produce. (For other reviews of this topic see ref. 89–93.) It is difficult to conceive how one can alter the function of a specific cell type by manipulating cyclic nucleotide metabolism without also changing the concentration of cyclic nucleotides in other cells and, consequently, altering their function as well. Fortunately, this problem may be overcome because the metabolism of the cyclic nucleotides is controlled by an extremely complex system of enzymes consisting of many isozymes and receptor subunits. Moreover, the characteristics of these enzyme systems differ between tissues and even between cell types (94). The development of pharmacological agents which could take advantage of these characteristic differences in the enzymes might result in the selective alteration of cyclic nucleotide metabolism only in the diseased tissue. To do this, one must first gain a firm understanding of the fundamental properties of these enzymes.

Adenylate Cyclase

Adenylate cyclase, the enzyme that catalyzes the conversion of ATP to cyclic AMP, consists of a catalytic subunit facing the interior of the cell and one or more receptors in contact with the intracellular fluid (97). (For reviews of this topic see ref. 6, 93, 95, 96.) The catalytic portion of adenylate cyclase of all mammalian cells is activated by sodium fluoride. Hormones and neurotransmitters, on the other hand, are thought to interact with specific receptor sites, present only on certain cell membranes. Thus, a given hormone activates only the adenylate cyclase contained in the

tissue in which the hormone exerts a physiological action, thereby explaining the specificity of hormonal actions (reviewed in 93).

Since variations in the properties of the receptor, rather than of the catalytic moiety of adenylate cyclase, apparently are responsible for the specificity with which different hormones can activate the various adenylate cyclases, one might be able to develop specific drugs that inhibit adenylate cyclase activity by preventing the hormone's interaction with the receptor. Unfortunately, little progress has been made in this promising area of research [see (93) for a more detailed discussion of agents that activate or inhibit adenylate cyclase].

Recently, a heat-stable, calcium-dependent protein, originally shown to increase phosphodiesterase activity (98), has been demonstrated to activate adenylate cyclase as well (99, 100). Whether this observation can be exploited to develop other types of selective agents for inhibiting specific adenylate cyclases must await further investigation. This endogenous activator of phosphodiesterase is discussed further in a subsequent section.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Guanylate Cyclase

Guanylate cyclase catalyzes the formation of cyclic GMP from GTP. (See ref. 93, 101, 102 for reviews.) This enzyme apparently exists in more than one form since guanylate cyclase activity isolated from soluble and particulate fractions migrate differently on gel filtration columns and respond differently to detergents and ions (103, 104).

The general, albeit greatly simplified, consensus relating the nucleotide cyclases to the function of the autonomic nervous system is that adenylate cyclase mediates β-adrenergic responses whereas guanylate cyclase is involved in cholinergic transmission. This is based largely on the results showing that sympathomimetic agents, as a rule, elevate cyclic AMP in adrenergically innervated structures (97, 105), whereas cholinergic agents cause an increase in the concentration of cyclic GMP (106, 107, 110).

Although it is well established that adenylate cyclase is activated by a wide variety of substances, only recently has it been demonstrated that guanylate cyclase can be stimulated in vitro (108, 109). However, there is substantial evidence that several hormones and neurotransmitters can increase the concentration of cyclic GMP (106, 107, 110, 111).

The development of agents that can selectively inhibit the activity of guanylate cyclase is still in its infancy but will surely progress as one gains a clearer understanding of the factors controlling the normal function of this enzyme. One important obstacle which must be overcome is the lack of hormonal responsiveness of guanylate cyclase in broken cell preparations.

Cyclic Nucleotide Phosphodiesterase

DISTRIBUTION Cyclic nucleotide phosphodiesterase activity is found in virtually all living cells, its relative activity varying greatly among different tissues (114, 115). (For reviews see ref. 93, 112, 113.) The regional and subcellular distribution of cyclic

GMP phosphodiesterase generally parallels that of cyclic AMP phosphodiesterase. Both enzyme activities are found in all subcellular fractions with most of the activity being in the 100,000 xg supernatant fluid (61, 114, 116–120).

Although phosphodiesterase is largely a soluble enzyme, some investigators have suggested that the particulate enzyme may be of greater physiological importance since the particulate fraction contains a high affinity form of the enzyme (112, 113, 121). However, the soluble fraction also contains a high affinity form of phosphodiesterase (94, 122–124). Moreover, it is not uncommon to achieve cyclic AMP concentrations of 10^{-4} M and more in whole tissue and cells in response to hormones (125–127). Thus, even if there were no intracellular compartmentalization of cyclic nucleotides, which in fact there is (128), it would not be difficult to achieve concentrations of cyclic nucleotides in at least the 0.1 millimolar range at the active site of phosphodiesterase. In our view, it is more likely that both the low and high K_m forms of phosphodiesterase play a role in regulating the intracellular concentration of cyclic nucleotides, the low K_m form of the enzyme being responsible for the basal levels and the high K_m form controlling the cyclic nucleotide concentrations following hormonal stimulation of the nucleotide cyclases (124).

SUBSTRATE SPECIFICITY The cyclic nucleotide phosphodiesterases are not completely substrate specific since some of the purified phosphodiesterase isozymes can hydrolyze cyclic AMP and cyclic GMP as well as a number of other cyclic nucleotides. However, the different isozymes of phosphodiesterase do have different relative affinities for the various cyclic nucleotides (129–137).

KINETIC PROPERTIES Phosphodiesterase exhibits anomalous kinetic behavior, suggesting either the existence of a single enzyme form with the property of negative cooperativity (138) or the existence of at least two forms of phosphodiesterase, one having a low affinity (high K_m) for cyclic AMP and the other having a high affinity (low K_m) (94, 124, 130, 139–143). The evidence suggests that both possibilities are true. On the one hand, it has been shown that there are different molecular forms of phosphodiesterase (see below) and, on the other hand, it has been shown that even highly purified isozymes of phosphodiesterase exhibit negative or positive cooperativity, suggesting that there are allosteric sites on the enzyme that influence the catalytic site (132, 138, 144, 145).

MULTIPLE MOLECULAR FORMS The existence of multiple molecular forms of phosphodiesterase has been demonstrated in many tissues by numerous investigators using a variety of techniques (94, 119, 125, 143, 146–156). The pattern and ratio of these phosphodiesterase isozymes vary with the specific tissue (94, 130, 148, 149, 156) and cell type (94, 157), and their activity can be altered both chronically and acutely. Chronically, it has been shown that a specific form of phosphodiesterase can be induced by treating astrocytoma cells with norepinephrine (157). Other studies have demonstrated that one of the phosphodiesterase isozymes increases with the age of the animals (143). Acutely, it has been demonstrated that the different phosphodiesterase isozymes can be selectively inhibited and activated by drugs (94,

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

120, 158–160). This phenomenon is discussed in detail in a later section and forms the basis of our hypothesis that drugs can be developed that selectively alter cyclic nucleotide metabolism in a diseased tissue.

ENDOGENOUS ACTIVATOR While purifying phosphodiesterase from heart, Cheung (98, 161) noticed that the phosphodiesterase activity decreased as the enzyme became more pure, suggesting that a factor that activates phosphodiesterase was being removed during the purification process (reviewed in 162). This activator has now been isolated and has been the subject of numerous investigations (137, 163–168). The activator is a heat-stable, calcium-dependent protein (149, 166, 169–171) with a molecular weight of between 11,000 and 40,000 (161, 172). The mechanism by which the activator increases phosphodiesterase activity is quite complex and is still controversial (171, 173–175). At low concentrations, the activator increases the maximum velocity of hydrolysis (V_{max}) of cyclic AMP phosphodiesterase (158, 173) and cyclic GMP phosphodiesterase (137), whereas at high concentrations the activator appears to decrease the apparent Michaelis constant (K_m) for cyclic AMP (137, 173, 174) and cyclic GMP (137).

This endogenous activator is of particular interest to us because it is extremely potent and highly selective. Less than 1 μg of activator is capable of increasing the activity of one of the molecular forms of phosphodiesterase more than tenfold, while the activity of other isozymes of phosphodiesterase isolated from the same tissue remains essentially unaltered (158). Moreover, this activable form of phosphodiesterase is not present in all tissues (60, 61, 120), and where it is found, it is present in different amounts relative to the other phosphodiesterase isozymes (149, 176). Therefore, by interfering with the activation process, one may be able to inhibit selectively phosphodiesterase activity in one tissue and not in another. Recent studies from our laboratory indicate that certain phenothiazine antipsychotics, in fact, do act by preventing the activation of phosphodiesterase (158, 159, 168).

The proposal that the interaction of the activator with the activable form of phosphodiesterase is controlled by a protein kinase–mediated release of activator from the membrane (177) suggests yet another site at which drugs might act to alter the metabolism of cyclic nucleotides.

To summarize, the properties of the cyclic nucleotide phosphodiesterase system pertinent to our discussion are the following: (*a*) the enzymes exist in several different molecular forms; (*b*) these isozymes are unequally distributed among the various tissues; (*c*) they have different kinetic properties and different substrate affinities; (*d*) they can be selectively activated by an endogenous protein; (*e*) they can be selectively inhibited by drugs; and (*f*) they possess allosteric sites that can influence their activity.

These properties of the cyclic nucleotide phosphodiesterases suggest to us that drugs could be developed which would interfere with phosphodiesterase activity through a variety of different mechanisms, namely: Drugs could act (*a*) by interfering with the binding of substrate to the active site of the enzyme by competing with the substrate for the active site, (*b*) by altering allosteric sites on the enzyme, or

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

(*c*) by interfering with the release or binding of activators. Drugs acting by these mechanisms may prove to be highly selective in modifying the phosphodiesterase activity and, consequently, the intracellular concentration of cyclic nucleotides in discrete cell types, and may, therefore, constitute a new class of pharmacologic agents useful in the treatment of diseases.

USE OF PHOSPHODIESTERASE INHIBITORS IN THE TREATMENT OF DISEASE

Drugs that inhibit phosphodiesterase activity have been used for decades in treating diseases [for recent reviews see (89, 120, 178)]. Although it is obvious that not all of the beneficial effects of these drugs can be explained by their inhibition of phosphodiesterase, nevertheless some drugs clearly act by inhibiting phosphodiesterase activity. The findings that several diseases are associated with an abnormal metabolism of cyclic nucleotides suggest that it might be possible to alter the course of diseases with specific phosphodiesterase inhibitors. We concentrate our discussion on the use of phosphodiesterase inhibitors only in those diseases in which there is good evidence of abnormalities in cyclic nucleotide metabolism.

Cancer Chemotherapeutic Agents

Of the many diseases associated with an abnormality in cyclic nucleotide physiology, neoplasia has received the most attention and offers one of the best examples of how, by controlling the metabolism of cyclic nucleotides, one might eventually alter the course of a disease (reviewed in 154, 179, 180). Generally, it has been found that malignant or transformed cells have a lower concentration of cyclic AMP when compared with normal or nontransformed cells (50, 181–184). Moreover, cyclic AMP or agents that increase the concentration of cyclic AMP, such as activators of adenylate cyclase or inhibitors or phosphodiesterase, reduce the growth rate of malignant cells (185–191), and, in some cases, revert the morphological and biochemical characteristics of these cells toward those of normal cells (192–195). Pertinent to our discussion are studies showing a correlation between phosphodiesterase inhibition and carcinostasis. For example, Hsie et al (196) have shown that dibutyryl cyclic AMP, which reduces the growth rate of transformed Chinese hamster ovary cells, also inhibits a low K_m phosphodiesterase in these cells. Tisdale & Phillips (197, 198) have demonstrated that the carcino
agents, such as chlorambucil and melphalan, may be due, at least in part, to their inhibition of phosphodiesterase. They demonstrated that chlorambucil increased the concentration of cyclic AMP only in those cell lines in which it inhibited cell growth; it had no effect on the concentration of cyclic AMP in chlorambucil-resistant cell lines. These results could be explained by the ability of alkylating agents to inhibit a low K_m form of phosphodiesterase; this form of the enzyme apparently is deficient in the drug-resistant cell lines.

Determination of the mechanism by which cyclic AMP reverts malignant cells toward the normal phenotype may provide the key to the pathogenesis of certain

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

forms of cancer. One clue into the mechanism by which cyclic AMP exerts its carcinostatic effects was derived from studies on mutant cells. Cyclic AMP failed to produce a carcinostatic effect in mutant lymphoma cells that lack a specific cyclic AMP-dependent protein kinase, suggesting that activation of protein kinase was essential for the growth-inhibitory effects of cyclic AMP (63, 64). Activation of protein kinase could, in turn, lead to the phosphorylation of histones (199) which could account for the increased DNA-mediated RNA synthesis (200), increased protein synthesis (201), and changes in the properties of the cell membrane (202, 203) seen in malignant cells treated with agents that increase the intracellular concentration of cyclic AMP.

Drugs Used in the Treatment of Psoriasis

Psoriasis is a disease of the skin characterized by an increased proliferation and a decreased differentiation of epidermal epithelium and by an increased accumulation of glycogen in these cells. Stimulated by the observations that cyclic AMP inhibits cellular proliferation (185), induces cellular differentiation (192–195), and stimulates the breakdown of glycogen (16), Voorhees and his co-workers (204, 205) have studied the concentration and metabolism of cyclic nucleotides in the skin of psoriatic patients. They demonstrated that the concentration of cyclic AMP was significantly decreased in psoriatic lesions (204), whereas the levels of cyclic GMP was increased (205). They also showed that β-adrenergic agonists such as isoproterenol inhibited epidermal cell division and that this effect was associated with a concomitant increase in cyclic AMP (206).

Studies of the adenylate cyclase system of psoriatic skin have not yielded conclusive results (207, 208). However, studies of the phosphodiesterase system showed a threefold increase in cyclic AMP phosphodiesterase activity in psoriatic lesions (208). The increased phosphodiesterase activity could explain the decreased concentrations of cyclic AMP and suggests that phosphodiesterase inhibitors may be effective clinical agents in treating psoriasis. This concept gains support from the recent clinical study showing that topically applied papaverine, a potent inhibitor of phosphodiesterase, was effective in the treatment of psoriasis (209).

Antiasthmatics

One suggestion concerning the etiology of asthma is that there is an intrinsic defect in the β-adrenergic receptor–adenylate cyclase complex. In the bronchioles this defect results in a decreased response to the smooth muscle relaxant effects of catecholamines (210). Support for this notion is derived from experiments showing that asthmatics have a diminished hyperglycemic (211) and lipolytic (212) response to epinephrine, and that leucocytes from these patients show a decreased accumulation of cyclic AMP in response to catecholamines (213). This hypothesis is supported further by studies showing that several agents that are effective in treating asthma, both acutely and prophylactically, increase the intracellular concentration of cyclic AMP. For example, the most effective agents for treating the acute asthmatic attack are epinephrine and isoproterenol, potent stimulators of adenylate cyclase. The phosphodiesterase inhibitor, theophylline, theophylline-containing

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

compounds, such as aminophylline, and several other phosphodiesterase inhibitors (214–216) are also effective in treating experimentally induced asthmatic reactions. Since theophylline often potentiates the effects of agents that stimulate adenylate cyclase activity in vitro (217), it would not be surprising to find that the concomitant administration of a catecholamine and theophylline is more effective than using either drug alone.

In individuals with allergic or intrinsic asthma, the asthmatic attack is precipitated by the binding of an allergen to a specific immunoglobulin on sensitized mast cells which results in the release of pharmacologically active substances such as histamine, bradykinin, and slow-reacting substances of anaphylaxis. The release of these agents appears to be controlled by the cyclic nucleotides since drugs that increase the intracellular concentration of cyclic AMP inhibit the antigen-induced release of histamine from sensitized tissues (218–220). In contrast, compound 48/80, which promotes the release of histamine, decreases the intracellular concentration of cyclic AMP (221), presumably by increasing phosphodiesterase activity (222).

A logical approach to the prophylactic treatment of asthma, therefore, would be through the use of drugs that would raise the intracellular levels of cyclic AMP in mast cells, thereby preventing the release of anaphylactoid mediators from these cells. The recently developed drug, sodium dichromoglycate, may act through this mechanism since it has been shown to be an inhibitor, albeit a weak one, of phosphodiesterase activity (223). Moreover, lymphocytes of patients receiving disodium chromoglycate do, in fact, have lower activities of phosphodiesterase than do those of untreated or theophylline-treated patients (224).

Certain steroids used prophylactically in treating asthma may also act by altering the metabolism or action of cyclic nucleotides. This would be predictable since glucocorticoids augment many physiological responses mediated by cyclic AMP, such as lipolysis and glycogenolysis (225). In this regard, hydrocortisone (226) and dexamethasone (227) have been reported to inhibit phosphodiesterase activity. However, since these agents are relatively weak phosphodiesterase inhibitors, they may be acting by increasing adenylate cyclase activity or by increasing the synthesis of a cyclic AMP–dependent-protein kinase.

Anti-Inflammatory Agents

The role of cyclic nucleotides in the inflammatory process has been reviewed recently by Ignarro et al (40). They and other investigators (228, 229) have demonstrated that cyclic AMP stabilizes lysosomal membranes, and cyclic GMP labilizes these membranes. Moreover, they have shown that the release of endogenous substances that mediate the inflammatory response is inhibited by agents which increase the intracellular concentration of cyclic AMP. Based on these results, one would predict that certain cyclic AMP phosphodiesterase inhibitors might have antiinflammatory properties. In fact, it has been shown that the antiinflammatory compounds, indomethacin and several of its analogues (230), arylaceticade, mefenemic acid, flufenic acid, quinolone compounds, and pyrazolones (230, 231) inhibit phosphodiesterase of chicken cartilage.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Drugs Used in the Treatment of Obesity

Cyclic AMP mediates the effect of catecholamines and other lypolytic agents (217). Evidence that some forms of obesity may be due to a defect in the metabolism of cyclic nucleotides comes from the study of Kupiecki & Adams (232) who showed that the adenylate cyclase system of adipocytes of genetically obese mice was poorly responsive to the stimulatory effects of catecholamines, and from that of Lovell-Smith & Sneyd (53) who showed an increased activity of phosphodiesterase in these mice as well as a decreased responsiveness of adenylate cyclase to catecholamines. This suggests that drugs which inhibit the specific phosphodiesterase isozyme in adipocytes may be of benefit in certain forms of obesity.

Drugs Used in the Treatment of Cardiovascular Disease

SMOOTH MUSCLE RELAXANTS Evidence for a role of cyclic AMP in the relaxation of smooth muscle has been reviewed by Somlyo et al (24). This evidence is based, in part, on the observations that several drugs that relax smooth muscle inhibit phosphodiesterase activity. These agents include papaverine (233, 234), isobutylmethylxanthine (235), diazoxide (236, 237), and chromonar (238). Wells et al (155, 160) have isolated two phosphodiesterase isozymes from porcine coronary arteries and have demonstrated that only one of the isozymes was activated by the endogenous protein activator. They also found that papaverine was a potent inhibitor of this activable isozyme whereas isobutylmethylxanthine was a more potent inhibitor of the nonactivable isozyme. Thus, as in the brain (see section on drugs used in the treatment of neuropsychiatric diseases), the isozymes of phosphodiesterase in coronary arteries can be selectively activated and inhibited by drugs. It would be important to learn whether the increased activity of phosphodiesterase seen in arteries of hypertensive animals (52) is due to a selective increase in one of these isozymes. If this were so, and one could find a drug that selectively inhibits this phosphodiesterase isozyme, a more potent and specific class of therapeutic agents for relaxing smooth muscle would evolve.

DIURETICS The hypothesis that cAMP is involved in diuresis is derived largely from experiments showing that cAMP increases the water permeability of toad bladder (239). The pharmacological evidence supporting this notion is based on the observations that clinically effective diuretics such as the benzothiadiazine derivatives inhibit phosphodiesterase activity (236, 237). Other classes of diuretics such as the mercurials (240), furosemide and bendroflumethiazide (89), ethacrynic acid, chlorthalidone, and acetazolamide (241) inhibit phosphodiesterase activity. However, since antidiuretic hormone, as well as diuretic agents, elevates cAMP, it is not readily apparent how inhibition of phosphodiesterase activity could explain the mechanism of action of diuretics. Perhaps this apparent contradiction could be clarified by determining the effects of all these agents on the specific phosphodiesterase isozymes in the cells on which these drugs are thought to act to produce their diuretic effects.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

CARDIAC GLYCOSIDES There is abundant evidence that cyclic AMP plays a role in the inotropic effects of the heart (26, 242). The observation that cardiac tissue from experimental animals and humans with congestive heart failure exhibits a reduced accumulation of cyclic AMP in response to glucagon (242–244) and norepinephrine (245) suggests that specific phosphodiesterase inhibitors might be of benefit in this disease. Support for this proposal was provided by Lippmann (246) who demonstrated that certain analogues of cardiac glycosides were more potent inhibitors of phosphodiesterase than was theophylline. In contrast, several naturally occurring and pharmacologically inactive glycosides were less potent inhibitors of this enzyme.

ANTIATHEROSCLEROTIC AGENTS The possible role of cyclic AMP in the pathogenesis of atherosclerosis has been reviewed by Shimamato (247). He suggested that a decreased concentration of cyclic AMP in arterial endothelial cells may be responsible for the accumulation of lipids in these cells. This accumulation of lipid and the development of atherosclerosis in rabbits was inhibited by dibutyryl cyclic AMP, and phthalazinol and its derivatives, agents that inhibit phosphodiesterase activity (247). The hypolipidemic agent, eritadenine [2(R)-dihydroxy-Y-(9-adenyl)-butyric acid], has also been shown to inhibit phosphodiesterase activity (248).

Oral Hypoglycemic Agents

Cyclic AMP induces the release of insulin from pancreatic islets (37). The therapeutic consequences, therefore, of inhibiting pancreatic phosphodiesterase would predictably be an increase in the release of insulin and a hypoglycemic effect. Accordingly, it was not surprising to find that several oral hypoglycemic agents such as the sulfonylureas inhibit phosphodiesterase activity (249–251). Agents that inhibit the dominant isozyme of phosphodiesterase present in pancreas might be still more effective and specific for treating adult onset diabetes.

Antiviral Agents

The antiviral agent, N-methyl-isatin-β semicarbazone, inhibited phosphodiesterase in peripheral blood lymphocytes but not in lymphoma cells (252). However, the role of cyclic nucleotides in viral infections is unclear.

Drugs Used in the Treatment of Neuropsychiatric Diseases

ANTIPSYCHOTIC AGENTS The effects of cyclic nucleotides in the central nervous system and their role in neuropsychiatric disorders have been reviewed recently (29). The pharmacologic evidence supporting a role for these cyclic nucleotides in the central nervous system is based largely on the actions of psychotropic drugs, such as the phenothiazine antipsychotics and butyrophenones, on the cyclic nucleotide system of brain. Apparently, these drugs have two distinct effects on the enzymes catalyzing the biosynthesis and hydrolysis of cyclic nucleotides. On the one hand, they inhibit specific norepinephrine- (253) and dopamine- (254) sensitive adenylate

cyclases, effects that would prevent the rise of cyclic AMP induced by these agents. On the other hand, they inhibit a specific activator-sensitive phosphodiesterase in brain (158, 159, 168), an effect that would tend to increase the intracellular concentration of cyclic nucleotides. Therefore, the net effect of these agents on the concentration of cyclic nucleotides in each area of the brain would depend on the relative quantity of the catecholamine-sensitive adenylate cyclases and the activator-sensitive phosphodiesterase in each of these brain areas. This theory predicts that antipsychotics might increase the level of cyclic AMP in one region of the brain and reduce it in another, an effect which, in fact, has recently been demonstrated (255).

The unusual specificity of antipsychotics for inhibiting a single phosphodiesterase isozyme in brain is discussed in more detail in a subsequent section.

ANTIANXIETY AGENTS Antianxiety agents such as the benzodiazepines have also been shown to inhibit phosphodiesterase of brain (168, 256). The effects of these drugs on the phosphodiesterase of several areas of cat brain varied with the specific brain area studied (257).

TRICYCLIC ANTIDEPRESSANTS Several dibenzazepines also inhibit phosphodiesterase activity. These agents were found to be competitive with cyclic AMP (256, 258, 259) and were more potent than theophylline (260), a finding which should not be viewed as remarkable since, despite its widespread use, theophylline is a relatively weak inhibitor of phosphodiesterase.

A FEASIBLE APPROACH TO THE SELECTIVE ALTERATION OF CYCLIC NUCLEOTIDE METABOLISM

Drugs that exert their effect by indiscriminately altering cyclic nucleotide metabolism would have little clinical utility since they would alter the function of many different types of cells. Ideally, the drug should be able to alter the concentration of specific cyclic nucleotides in a diseased tissue without substantially affecting the concentration of these cyclic nucleotides in normal tissue.

We feel that there is a good possibility of realizing this ideal. We base our optimism on the following observations: there are different patterns and ratios of the phosphodiesterase isozymes in different tissues; these isozymes can be selectively altered by drugs; certain diseases are associated with an abnormal metabolism of cyclic nucleotides; and drugs that alter the intracellular concentrations of cyclic nucleotides already have been shown to be fairly selective and effective therapeutic agents.

Some of the evidence in support of these statements has already been reviewed briefly. A more detailed discussion of the distribution and selective inhibition of the phosphodiesterase isozymes is presented below.

Several studies have shown that drugs could differentially inhibit the phosphodiesterase activity of different tissues (89, 90, 253, 259, 261, 262). For example, Uzunov & Weiss (253) showed that trifluoperazine was more effective in inhibiting

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

the phosphodiesterase of cerebrum than that of cerebellum, and Pichard et al (259) demonstrated that dipyridamole, an inhibitor of platelet aggregation, is a more potent inhibitor of the phosphodiesterase of platelets than that of the brain, while the tricyclic antidepressants, opipramol, nortryptyline, and imipramine were more potent inhibitors of the phosphodiesterase of brain than that of platelets. These and other studies suggested that the selectivity of the phosphodiesterase inhibitors was due to a difference in the distribution or in the sensitivity of the phosphodiesterase isozymes to inhibitors.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Distribution of Phosphodiesterase Isozymes

It is now clear that there are marked differences in the distribution of the various cyclic nucleotide phosphodiesterase isozymes among different tissues and cell types (see section on multiple molecular forms). For example, the rat cerebellum was found to have six forms of phosphodiesterase (149), cerebrum four forms (94), and caudate nucleus two forms (119). The lung (120) also had two forms of phosphodiesterase activity but the types and ratio of these isozymes were different from those of the caudate nucleus. A cloned astrocytoma cell line (C21) had two forms of phosphodiesterase as well, but the type and ratio of these isozymes was unlike that found in the caudate nucleus or in lung (94). Finally, cloned neuroblastoma cells were shown to have a single isozyme of phosphodiesterase, and this isozyme was different from either of the two forms found in the C21 astrocytoma cell lines (94).

Selective Activation and Inhibition of the Phosphodiesterase Isozymes

The other important property of these isozymes that must be satisfied before phosphodiesterase inhibitors can be clinically useful is that they must be selectively activated or inhibited by drugs. Studies from our laboratory demonstrated that some of the phosphodiesterase isozymes can, in fact, be selectively inhibited and activated by endogenous and exogenous agents (94, 119, 120, 144, 149, 158, 159), a finding that has recently been confirmed by other laboratories (160, 175). For example, of the four isozymes of phosphodiesterase isolated from the rat cerebrum (94) (isozymes were designated as Peaks I to IV according to the order of their emergence from a preparative polyacrylamide gel column), Peak I, which hydrolyzes cyclic AMP and not cyclic GMP, is relatively resistant to the commonly used inhibitors of phosphodiesterase (120, 158, 159). Peak II, which is the major form of phosphodiesterase in cerebrum (94, 158, 159), is of great pharmacological and biological interest, since it is the only major isozyme which is activated by the endogenous activator of phosphodiesterase (94, 158). This isozyme, which is sensitive to the inhibitory effects of cyclic GMP and is particularly sensitive to the effects of trifluoperazine and other antipsychotic agents when it is in the activated form, has been examined in some detail (94, 137, 158, 168; see below). Peak III phosphodiesterase was not significantly activated by the protein activator and was relatively sensitive to theophylline and papaverine (158). Peak IV phosphodiesterase was more sensitive to papaverine than to trifluoperazine or theophylline (158). A summary of these studies is presented in Table 2.

Table 2 Selective inhibition of the multiple forms of cyclic AMP phosphodiesterase of rat cerebrum[a]

Peak	Ki values (μM)			
	Theophylline	Trifluoperazine	Papaverine	Cyclic GMP
I	2000	1000	180	730
II (not activated)	350	250	100	20
II (activated)	350	10	100	10
III	180	250	60	25
IV	600	75	25	

[a]The phosphodiesterase isozymes were prepared from the soluble 100,000 × *g* supernatant fraction of rat cerebrum. Each peak of phosphodiesterase was identified by its electrophoretic mobility on a polyacrylamide gel column (149). Phosphodiesterase activity was determined by the luciferin-luciferase technique (140). The Peak II isozyme was assayed in the absence and presence of optimum amounts of the calcium-dependent activator. The activator produced approximately a tenfold increase in phosphodiesterase activity. (Taken in part from 158.)

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Mechanism for the Selective Inhibition of the Phosphodiesterase Isozymes

One obvious explanation for the selective inhibition of the phosphodiesterase isozymes is that the phosphodiesterase inhibitors are acting by different mechanisms. Therefore, considerable effort was spent in studying the mechanism of action of these agents. Thus far, evidence has been obtained for four distinct mechanisms of action. The drugs may act (*a*) by competing with the cyclic nucleotide substrate; (*b*) by acting at the substrate site noncompetitively; (*c*) by acting at an allosteric site to increase or decrease the affinity of the enzyme for its substrate; and (*d*) by acting on one of the endogenous activators or cofactors for the enzyme.

Methylxanthines, like theophylline, which have a structural similarity to the cyclic nucleotides, competitively inhibit phosphodiesterase. This was shown initially in cardiac tissue (115) and more recently in isolated phosphodiesterase isozymes of brain (94, 158). Low concentrations of papaverine also competitively inhibit the phosphodiesterase isozymes (158), whereas high concentrations appear to act noncompetitively (158). Other phosphodiesterase inhibitors such as SQ 20,009 also have been reported to act both competitively and noncompetitively (262).

A compound that apparently acts at an allosteric site to alter the activity of cyclic AMP phosphodiesterase is the cyclic nucleotide, cyclic GMP (132, 144, 145). This latter observation suggests that the concentration of one cyclic nucleotide may influence the metabolism of another cyclic nucleotide.

The final mechanism by which compounds may act is by interfering with the endogenous activator of phosphodiesterase. This mechanism may prove to be one of the most specific since the activity of only one major form of phosphodiesterase is increased by this endogenous activator (149, 158–160, 175). Since trifluoperazine was a particularly effective inhibitor of the Peak II isozyme of phosphodiesterase, and since this is the only major phosphodiesterase isozyme which is activated by

the endogenous activator of phosphodiesterase, we examined the influence of trifluoperazine on Peak II in the presence and absence of the phosphodiesterase activator. The phenothiazine was found to be 25 times more potent an inhibitor of activated Peak II than of the unactivated form (Table 2). Moreover, this inhibition of phosphodiesterase by trifluoperazine (158) or chlorpromazine (168) could be overcome by adding excess activator; kinetic analysis of these data revealed that these phenothiazines were competitive inhibitors of the activation of Peak II phosphodiesterase. Other centrally acting drugs were also shown to be highly specific inhibitors of the activated form of phosphodiesterase (169).

The specific mechanisms by which phenothiazine antipsychotics block the activation of phosphodiesterase has recently been studied in our laboratory where it was shown that the phenothiazines act by binding to the endogenous activator (R. Levin and B. Weiss, unpublished). Since the same protein that increases phosphodiesterase activity apparently is responsible for the activation of adenylate cyclase, our results may explain why phenothiazine antipsychotics inhibit the activity of both adenylate cyclase (253, 254, 263) and phosphodiesterase.

These results suggest again that drugs, by selectively inhibiting the phosphodiesterase isozymes, may be able to raise the intracellular concentration of cyclic AMP in discrete tissues. For example, phenothiazine antipsychotics should raise the concentration of cyclic AMP in cerebral cortex, which has a high proportion of Peak II phosphodiesterase (94, 158), but should have little effect on peripheral tissues that have little or none of this isozyme (60, 61, 144). Papaverine, on the other hand, may exert a greater effect on tissues with a high proportion of Peak III or Peak IV phosphodiesterase. Studies of this type might also yield compounds that exert a selective inhibitory action on either the cyclic GMP phosphodiesterase or cyclic AMP phosphodiesterase (160), a result which would render even greater specificity of action to these compounds.

Possible Clinical Application of the Selective Inhibition of Phosphodiesterase Isozymes

Although the extrapolation of these basic studies to therapeutic applications is a long way off, the goal is clear, that is, to find drugs that will selectively inhibit or activate the major isozyme of phosphodiesterase in each tissue. If a disease is caused by a defect in the metabolism of cyclic nucleotides, these drugs may be able to restore the concentration of cyclic nucleotides in that tissue without adversely affecting other tissue.

Recently, we have begun to test this approach in an attempt to alter selectively the cyclic nucleotide metabolism of malignant cells. The investigations to be described were based on the assumptions that (*a*) an abnormality in cyclic nucleotide physiology was a characteristic and perhaps a causative factor in neoplastic disease; (*b*) the major isozyme of malignant tissue may be different from that of normal tissues; (*c*) this abnormal metabolism might be corrected by the selective manipulation of phosphodiesterase isozymes; (*d*) this isozyme could be selectively manipulated by drugs; and (*e*) the correction of cyclic nucleotide metabolism would result in the restoration of normal growth and differentiation of neoplastic tissue and would, therefore, provide a new therapeutic approach to malignant disease.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

For these studies we chose the murine leukemias, L5178Y and L1210, because there was substantial evidence to suggest that these and other leukemic lymphocytes had an abnormal cyclic nucleotide system (see Table 1). For example, Monahan and co-workers (264) demonstrated that unlike normal cells, which accumulate cyclic AMP at high cell density and exhibit contact inhibition of growth, L5178Y cells do not. The addition of exogenous cyclic AMP to these cells, however, does inhibit their growth (187). Our studies showing that both L5178Y and L1210 leukemic lymphocytes had approximately a tenfold increase in activity in both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase compared with that of normal lymphocytes (59, 60, 61) provided one explanation for the inability of cyclic AMP to accumulate in these leukemic lymphocytes.

To determine whether this increased phosphodiesterase activity of leukemic lymphocytes was due to a specific elevation of one of the phosphodiesterase isozymes, we separated the phosphodiesterases of these lymphocytes by gel electrophoresis according to the procedure of Uzunov & Weiss (149). Three major forms of phosphodiesterase were found. One peak, which constituted about 5% of the total activity, was found in fractions 30–35; a second peak, also having about 5% of the activity, was in fractions 40–50; the third peak (fractions 80–100) accounted for about 90% of the activity recovered from the gel electrophoresis column. This pattern of phosphodiesterase activity was clearly different from that of all other tissues we have studied thus far. For example, leukemic lymphocytes do not contain the major form of phosphodiesterase found in most brain areas, i.e. the activator-sensitive Peak II, nor do they have substantial quantities of the isozyme which is the predominant form found in salivary gland. Thus, the mouse salivary gland had only two major forms of phosphodiesterase activity. The first peak (fractions 40–50) accounted for about 60% of the activity and the second peak (fractions 60–70) for about 40% of the recovered activity. The different isozymic pattern of phosphodiesterase activity of leukemic lymphocytes suggested that the phosphodiesterase in this tissue might be selectively inhibited by drugs.

To test this hypothesis we compared the effects of a phenothiazine (chlorpromazine), a methylxanthine (isobutylmethylxanthine), a benzylisoquinolone (papaverine), and a pyrazole derivative (SQ 20,009) on the phosphodiesterase activity of cerebrum, salivary gland, and leukemic lymphocytes. As predicted from earlier experiments, the phosphodiesterase of cerebrum, which is rich in the Peak II isozyme, was very sensitive to the inhibitory effects of chlorpromazine ($I_{50} = 30\ \mu M$) whereas the phosphodiesterase of leukemic lymphocytes, which is essentially devoid of Peak II phosphodiesterase, was totally resistant to the phenothiazine (61). Papaverine, which is a potent inhibitor of Peak III phosphodiesterase of cerebrum (159), produced a marked inhibition of the major phosphodiesterase isozyme of leukemic lymphocytes. SQ 20,009, a drug which also has been shown to be a selective inhibitor of isolated phosphodiesterase isozymes (120), was 10 times more potent an inhibitor of the major form of phosphodiesterase of salivary gland than that of leukemic lymphocytes (Figure 1). In contrast, isobutylmethylxanthine was a more specific inhibitor of the major phosphodiesterase isozyme of leukemic lymphocytes than that of the major isozyme of salivary gland (Figure 2). These studies,

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

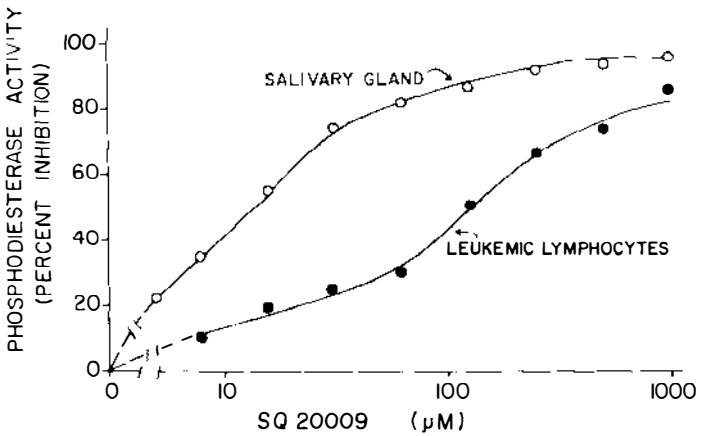

Figure 1 Effect of SQ 20,009 on purified isozymes of phosphodiesterase from mouse salivary gland and leukemic lymphocytes. Tissue was homogenized in 50 mM glycylglycine buffer, pH 8.0, and centrifuged at 100,000 xg for 60 min. The phosphodiesterase isozymes in the supernatant fraction were separated by preparative polyacrylamide gel electrophoresis (149). The activity of phosphodiesterase was assayed by the firefly luciferin-luciferase technique (140) with 100 μM cyclic AMP as substrate. The figure shows the effects of varying concentrations of SQ 20,009 [1-ethyl-4-(isopropylidenehydrazino)-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester] on the major phosphodiesterase isozyme isolated from salivary gland (fractions 40–50) and leukemic lymphocytes (fractions 80–100).

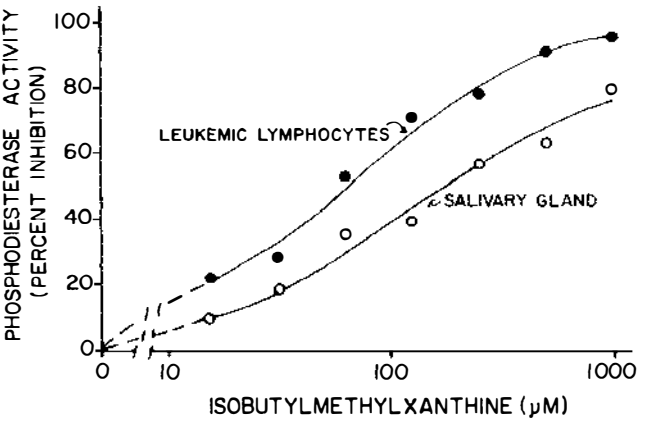

Figure 2 Effect of isobutylmethylxanthine on purified isozymes of phosphodiesterase from mouse salivary gland and leukemic lymphocytes. The tissue and isozymes were prepared as described in the legend to Figure 1. The major phosphodiesterase isozymes of salivary glands and leukemic lymphocytes were assayed in the absence and presence of varying concentrations of isobutylmethylxanthine.

combined with the evidence that cyclic AMP is cytotoxic to leukemic lymphocytes (63, 64), suggest that the development of selective inhibitors of the major phosphodiesterase isozyme of leukemic lymphocytes, may yield new agents for treating certain forms of leukemia.

Moreover, these studies support the hypothesis that by identifying and characterizing the major form of phosphodiesterase in each tissue, it may be possible to predict which drug would selectively inhibit the phosphodiesterase of that tissue. Further studies on the mechanisms by which these agents act and on the structure-activity relationship of these drugs will surely lead to more potent and more selective inhibitors of the individual phosphodiesterase isozymes.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

SUMMARY

The cyclic nucleotides, cyclic AMP and cyclic GMP, influence a wide variety of biological functions, and many diseases apparently are associated with or may even be caused by an abnormal intracellular concentration of these cyclic nucleotides. This abnormal concentration of cyclic AMP or cyclic GMP in diseased tissue has been shown to be due to an alteration in the enzymes that catalyze their synthesis (nucleotide cyclases) or hydrolysis (cyclic nucleotide phosphodiesterases). Accordingly, by correcting the defect in cyclic nucleotide metabolism, the course of a disease may be favorably altered.

The cyclic nucleotide phosphodiesterases, which hydrolyze cyclic AMP and cyclic GMP, are instrumental in controlling the concentration of these cyclic nucleotides. Since these enzymes exist as a complex system of isozymes having characteristic differences among tissues, they represent excellent targets for selective pharmacological manipulation. Recent studies showed that the individual isozymes can be selectively activated and inhibited and that this selective alteration of enzyme activity is due to differences in the mechanisms by which these agents act. For example, methylxanthines inhibit phosphodiesterase activity by competing with the cyclic nucleotide substrates, SQ 20,009 inhibits phosphodiesterase activity both competitively and noncompetitively, cyclic GMP activates cyclic AMP phosphodiesterase by binding to an allosteric site, and the phenothiazine antipsychotics inhibit a specific isozyme of phosphodiesterase by interfering with an endogenous protein activator of this phosphodiesterase isozyme.

Since each tissue has its own peculiar pattern and ratio of the phosphodiesterase isozymes, drugs may be developed that will inhibit the major isozyme in each tissue, resulting in the selective alteration of the concentration of cyclic nucleotides in this tissue. As an example, our studies showed that leukemic lymphocytes have a markedly increased activity of phosphodiesterase compared with that of normal lymphocytes. This increase was due to an elevation of one of the phosphodiesterase isozymes. Since this isozyme can be inhibited by drugs which have relatively little action on the major phosphodiesterase isozymes of other tissue, one would predict that the concentration of cyclic nucleotides would be elevated in the leukemic cells but not in normal tissue. And since malignant cell growth can be repressed by increasing the intracellular concentration of cyclic AMP, specific phosphodiesterase

inhibitors may inhibit the growth and induce the differentiation in these leukemic lymphocytes without adversely affecting the function of other tissue.

Thus, the evidence reviewed in this paper favors the conclusion that the determination of the biochemical properties of the phosphodiesterase isozymes in normal and diseased tissue and the continued search for new and more selective inhibitors of each phosphodiesterase isozyme may lead to the development of a new class of selective therapeutic agents, ones that act by altering the metabolism of cyclic nucleotides.

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

ACKNOWLEDGMENT

This investigation was supported in part by Grant Number CA15883, awarded by the National Cancer Institute, DHEW. We thank Ms. J. S. Siew for her help in typing the manuscript.

Literature Cited

1. Weiss, B. 1970. In *Biogenic Amines as Physiological Regulators,* ed. J. J. Blum, 35–73. New Jersey: Prentice-Hall
2. Greengard, P., Costa, E., eds. 1970. *Adv. Biochem. Psychopharmacol.* 3:11–381
3. Weiss, B., Crayton, J. W. 1970. *Adv. Biochem. Psychopharmacol.* 3:217–39
4. Robison, G. A., Butcher, R. W., Sutherland, E. W. 1971. *Cyclic AMP.* New York: Academic
5. Hardman, J. G., Robison, G. A., Sutherland, E. W. 1971. Cyclic nucleotides. *Ann. Rev. Physiol.* 33:311–36
6. Weiss, B. 1971. Cyclic AMP and cell function. *Ann. NY Acad. Sci.* 185: 507–19
7. Hittelman, K. J., Butcher, R. W. 1972. In *Effects of Drugs on Cellular Control Mechanisms,* ed. B. R. Rabin, R. B. Freedman, 153–74. Baltimore: Univ. Park Press
8. Greengard, P., Paoletti, R., Robison, G. A., eds. 1972. *Adv. Cyclic Nucleotide Res.,* Vol. 1. 590 pp.
9. Rall, T. 1972. Role of adenosine 3',5'-monophosphate (cyclic AMP) in actions of catecholamines. *Pharmacol. Rev.* 24:399–409
10. Bitensky, M. W., Gorman, R. E. 1973. Cellular responses to cyclic AMP. *Prog. Biophys. Mol. Biol.* 26:411–61
11. Kahn, R. H., Lands, W. E. M., eds. 1973. *Prostaglandins and Cyclic AMP: Biological Actions and Clinical Applications.* New York: Academic. 304 pp.
12. Greengard, P., Robison, G. A., eds. 1973. *Adv. Cyclic Nucleotide Res.,* Vol. 3. 383 pp.
13. Greengard, P., Robison, G. A. eds. 1974. *Adv. Cyclic Nucleotide Res.,* Vol. 4. 461 pp.
14. Braun, W., Lichtenstein, L. M., Parker, C. W., eds. 1974. *Cyclic AMP, Cell Growth and the Immune Response.* New York: Springer. 411 pp.
15. Drummond, G. I., Greengard, P., Robison, G. A., eds. 1975. *Adv. Cyclic Nucleotide Res.,* Vol. 5. 841 pp.
16. Robison, G. A., Butcher, R. W., Sutherland, E. W. 1968. Cyclic AMP. *Ann. Rev. Biochem.* 37:149–74
17. Weiss, B., Kidman, A. D. 1969. *Adv. Biochem. Psychopharmacol.* 1:131–64
18. Ryan, W. L., Heidrick, M. L. 1968. Inhibition of cell growth in vitro by adenosine 3',5'-monophosphate. *Science* 162:1484–85
19. Castañeda, M., Tyler, A. 1968. Adenyl cyclase in plasma membrane preparations of sea urchin eggs and its increase in activity after fertilization. *Biochem. Biophys. Res. Commun.* 33:782–87
20. Zalin, R. J., Montague, W. 1975. Changes in cyclic AMP, adenylate cyclase and protein kinase levels during the development of embryonic chick skeletal muscle. *Exp. Cell Res.* 93: 55–62
21. Bottomley, S. S., Whitcomb, W. H., Smithee, G. A., Moore, M. Z. 1971. Effect of cyclic adenosine 3',5'-monophosphate on bone marrow Δ-aminolevulinic acid synthetase and erythrocyte iron uptake. *J. Lab. Clin. Med.* 77:793–801
22. Rodgers, G. M., Fisher, J. W., George, W. J. 1975. Renal cyclic AMP accumulation and adenylate cyclase stimulation

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

by erythropoietic agents. *Am. J. Physiol.* 229:1387–92
23. Norris, A., Gorshein, D., Besa, E. C., Leonard, R. A., Gardner, F. H. 1974. Stimulation and suppression of erythropoiesis in the plethoric mouse by cyclic nucleotides. *Proc. Soc. Exp. Biol. Med.* 145:975–78
24. Somylo, A. P., Somlyo, A. V., Smiesko, V. 1972. *Adv. Cyclic Nucleotide Res.* 1:175–94
25. Bar, H. P. 1974. *Adv. Cyclic Nucleotide Res.* 4:195–237
26. Kukovetz, W., Poch, G. 1972. *Adv. Cyclic Nucleotide Res.* 1:261–90
27. Entman, M. L., Levey, G. S., Epstein, S. E. 1969. Mechanism of action of epinephrine and glucagon on the canine heart: Evidence for increase in sarcotubular calcium stores mediated by cyclic 3',5'-AMP. *Circ. Res.* 25:429–38
28. Mayer, S. E., Dobson, J. G., Gross, S. R., Khoo, J. C., Steinberg, D. 1975. In *Cyclic Nucleotides in Disease,* ed. B. Weiss, 105–16. Baltimore: Univ. Park Press
29. Weiss, B., Greenberg, L. H. 1975. See Ref. 28, pp. 269–320
30. Greengard, P., McAfee, D. A., Kebabian, J. W. 1972. *Adv. Cyclic Nucleotide Res.* 1:337–56
31. Bitensky, M. W., Miller, W. H., Gorman, R. E., Neufeld, A. H., Robinson, R. 1972. *Adv. Cyclic Nucleotide Res.* 1:317–36
32. Singh, J. N., Dhalla, N. S. 1975. *Adv. Cyclic Nucleotide Res.* 5:759–70
33. Parker, C. W., Sullivan, T. J., Wedner, H. J. 1974. *Adv. Cyclic Nucleotide Res.* 4:1–79
34. Marsh, J. M., Butcher, R. W., Savard, K., Sutherland, E. W. 1966. The stimulatory effect of luteinizing hormone on adenosine 3',5'-monophosphate accumulation in corpus luteum slices. *J. Biol. Chem.* 241:5436–40
35. Malaisse, W. J., Pipeleers, D. G., Levy, J. 1974. The stimulus-secretion coupling of glucose-induced insulin release. XVI. A glucose-like and calcium-independent effect of cyclic AMP. *Biochim. Biophys. Acta* 362:121–28
36. Turtle, J. R., Littleton, G. K., Kipnis, D. M. 1967. Stimulation of insulin secretion by theophylline. *Nature* 213:727–28
37. Kuo, J. F., Kuo, W-N. 1975. See Ref. 28, pp. 211–26
38. Sullivan, T. J., Parker, K. L., Stenson, W., Parker, C. W. 1975. Modulation of cyclic AMP in purified rat mast cells. I. Responses to pharmacologic, metabolic, and physical stimuli. *J. Immunol.* 114:1473–79
39. Sullivan, T. J., Parker, K. L., Eisen, S. A., Parker, C. W. 1975. Modulation of cyclic AMP in purified rat mast cells. II. Studies on the relationship between intracellular cyclic AMP concentrations and histamine release. *J. Immunol.* 114:1480–85
40. Ignarro, L. J. 1975. See Ref. 28, pp. 184–210
41. Goldberg, N. D., Haddox, M. K., Nicol, S. E., Glass, D. B., Sanford, C. H., Kuehl, F. A. Jr., Estensen, R. 1975. *Adv. Cyclic Nucleotide Res.* 5:307–30
42. Fichman, M. P., Brooker, G. 1972. Deficient renal cyclic AMP production in nephrogenic diabetes insipidus. *J. Clin. Endocrinol. Metab.* 35:35–47
43. Dousa, T. P., Rowland, R. G., Carone, F. A. 1973. Renal medullary adenylate cyclase in drug-induced nephrogenic diabetes insipidus. *Proc. Soc. Exp. Biol. Med.* 142:720–22
44. Burk, R. R., 1968. Reduced adenyl cyclase activity in a polyoma virus transformed cell line. *Nature* 219:1272–75
45. Weiss, B., Shein, H. M., Snyder, R. 1971. Adenylate cyclase and phosphodiesterase activity of normal and SV_{40} virus-transformed hamster astrocytes in cell culture. *Life Sci.* 10:1253–60
46. Allen, D. O., Munshower, J., Morris, H. P., Weber, G. 1971. Regulation of adenyl cyclase in hepatomas of different growth rates. *Cancer Res.* 31:557–60
47. Ney, R. L., Hochella, N. J., Grahame-Smith, D. G., Dexter, R. N., Butcher, R. W. 1969. Abnormal regulation of adenosine 3',5'-monophosphate and corticosterone formation in an adrenocortical carcinoma. *J. Clin. Invest.* 48:1733–39
48. Macchia, V., Meldolesi, M. F., Chiariello, M. 1972. Adenyl-cyclase in a transplantable thyroid tumor: Loss of ability to respond to TSH. *Endocrinology* 90:1483–91
49. Polgar, P., Vera, J. C., Kelley, P. R., Rutenburg, A. M. 1973. Adenylate cyclase activity in normal and leukemic human leukocytes as determined by a radioimmunoassay for cyclic AMP. *Biochim. Biophys. Acta* 297:378–83
50. Kemp, R. G., Hsu, P-Y., Duquesnoy, R. J. 1975. Changes in lymphoid cyclic adenosine 3':5'-monophosphate metabolism during murine leukemogenesis. *Cancer Res.* 35:2440–45

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

51. Nesbitt, J. A. III, Anderson, W. B., Miller, Z., Pastan, I., Russell, T. R., Gospodarowicz, D. 1976. Guanylate cyclase and cyclic guanosine 3':5'-monophosphate phosphodiesterase activities and cyclic guanosine 3':5'-monophosphate levels in normal and transformed fibroblasts in culture. *J. Biol. Chem.* 251:2344–52
52. Amer, M. S. 1973. Cyclic adenosine monophosphate and hypertension. *Science* 179:807–9
53. Lovell-Smith, C. J., Sneyd, J. G. T. 1974. Lipolysis and adenosine 3',5'cyclic monophosphate in adipose tissue of the New Zealand obese mouse; the activities of adipose tissue adenyl cyclase and phosphodiesterase. *Diabetologia* 10:655–59
54. Kupiecki, F. P. 1969. Reduced adenosine 3',5'-monophosphate phosphodiesterase activity in the pancreas and adipose tissue of spontaneously diabetic mice. *Life Sci.* 8:645–49
55. Hemington, J. G., Chenoweth, M., Dunn, A. 1973. Cyclic nucleotide phosphodiesterase activity in the plasma and erythrocytes of normal and diabetic rats. *Biochim. Biophys. Acta* 304: 552–59
56. Das, I. 1973. Effect of diabetes and insulin on the rat heart adenyl cyclase, cyclic AMP phosphodiesterase and cyclic AMP. *Hormone Metab. Res.* 5:330–33
57. Schmidt, S. Y., Lolley, R. N. 1973. Cyclic-nucleotide phosphodiesterase: An early defect in inherited retinal degeneration of C3H mice. *J. Cell Biol.* 57:117–23
58. Farber, D. B., Lolley, R. N. 1973. Proteins in the degenerative retina of C3H mice: Deficiency of a cyclic-nucleotide phosphodiesterase and opsin. *J. Neurochem.* 21:817–28
59. Hait, W. N., Weiss, B. 1975. Increased activity of cyclic AMP and cyclic GMP phosphodiesterase in lymphocytic leukemia. *Clin. Res.* 23:595A
60. Hait, W. N., Weiss, B. 1976. Increased cyclic nucleotide phosphodiesterase activity in leukemic lymphocytes. *Nature* 259:321–23
61. Hait, W. N., Weiss, B. 1977. Characterization of the cyclic nucleotide phosphodiesterases of normal and leukemic lymphocytes. *Biochim. Biophys. Acta.* In press
62. Drezner, M., Neelon, F. A., Lebovitz, H. E. 1973. Pseudohypoparathyroidism Type II: A possible defect in the reception of the cyclic AMP signal. *N. Engl. J. Med.* 289:1056–60
63. Daniel, V., Litwack, G., Tomkins, G. M. 1973. Induction of cytolysis of cultured lymphoma cells by adenosine 3',5'-cyclic monophosphate and the isolation of resistant variants. *Proc. Natl. Acad. Sci. USA* 70:76–79
64. Coffino, P., Bourne, H. R., Tomkins, G. M. 1975. Mechanism of lymphoma cell death induced by cyclic AMP. *Am. J. Pathol.* 81:199–204
65. Goldberg, M. L., Burke, G. C., Morris, H. P. 1975. Cyclic AMP and cyclic GMP content and binding in malignancy. *Biochem. Biophys. Res. Commun.* 62:320–27
66. Criss, W. E., Morris, H. P. 1973. Protein kinase activity in Morris hepatomas. *Biochem. Biophys. Res. Commun.* 54:380–86
67. Prasad, K. M., Fogleman, D., Gaschler, M., Sinha, P. K., Brown, J. L. 1976. Cyclic nucleotide-dependent protein kinase activity in malignant and cyclic AMP-induced "differentiated" neuroblastoma cells in culture. *Biochem. Biophys. Res. Commun.* 68:1248–55
68. Zapf, J., Waldvogel, M., Froesch, E. R. 1973. Protein kinase and cyclic AMP-binding activities in liver and adipose tissue of normal, streptozotocin-diabetic and adrenalectomized rats. *FEBS Lett.* 36:253–56
69. Murad, F. 1973. *Adv. Cyclic Nucleotide Res.* 3:355–87
70. Tucci, J. R., Lin, T., Kopp, L. E. 1973. Urinary cyclic 3',5'-adenosine monophosphate levels in diabetes mellitus before and after treatment. *J. Clin. Endocrinol. Metab.* 37:832–35
71. Lin, T., Kopp, L. E., Tucci, J. R. 1973. Urinary excretion of cyclic 3',5'-adenosine monophosphate in hyperthyroidism. *J. Clin. Endocrinol. Metab.* 36:1033–36
72. Guttler, R. B., Shaw, J. W., Otis, C. L., Nicoloff, J. T. 1975. Epinephrine-induced alterations in urinary cyclic AMP in hyper- and hypothyroidism. *J. Clin. Endocrinol. Metab.* 41:707–11
73. Murad, F., Pak, C. 1972. Urinary excretion of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate. *N. Engl. J. Med.* 286:1832–37
74. Schmidt-Gayk, H., Roher, H. D. 1973. Urinary excretion of cyclic adenosine monophosphate in the detection and diagnosis of primary hyperparathyroidism. *Surg. Gynecol. Obstet.* 137: 439–44

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

75. Chase, L. R., Melson, G. L., Aurbach, G. D. 1969. Pseudohypoparathyroidism: Defective excretion of 3',5'-AMP in response to parathyroid hormone. *J. Clin. Invest.* 48:1832–44
76. Chase, L. R., Aurbach, G. D. 1967. Parathyroid function and the renal excretion of 3',5'-adenylic acid. *Proc. Natl. Acad. Sci. USA* 58:518–25
77. Bell, N. H., Clark, C. M., Avery, S., Sinha, T., Trygstad, C. W., Allen, D. O. 1974. Demonstration of a defect in the formation of adenosine 3',5'-monophosphate in vasopressin-resistant diabetes insipidus. *Pediatr. Res.* 8:223–30
78. Fireman, P. 1973. Metabolic abnormalities in asthma-decreased urinary cyclic AMP. *Int. Arch. Allergy Appl. Immunol.* 45:123–27
79. Paul, M. I., Ditzion, B. P., Pauk, G. L., Janowsky, D. S. 1970. Urinary adenosine 3',5'-monophosphate excretion in affective disorders. *Am. J. Psychiatry* 126:1493–97
80. Abdulla, Y. H., Hamadah, K. 1970. 3',5'-Cyclic adenosine monophosphate in depression and mania. *Lancet* 1:378–81
81. Paul, M. I., Cramer, H., Bunney, W. E. Jr. 1971. Urinary adenosine 3',5'-monophosphate in the switch process from depression to mania. *Science* 171:300–3
82. Jenner, F. A., Sampson, G. A., Thompson, E. A., Sommerville, A. R., Beard, N. A., Smith, A. A. 1972. Manic depressive psychosis and urinary excretion of cyclic AMP. *Br. J. Psychiatry* 121:236–37
83. Brown, B. C., Salway, J. G., Albano, J. P., Hullin, R. P., Ekins, R. P. 1972. Urinary excretion of cyclic AMP and manic-depressive psychosis. *Br. J. Psychiatry* 120:405–8
84. Murad, F., Kimura, H., Hopkins, H. A., Looney, W. B., Kovacs, C. J. 1975. Increased urinary excretion of cyclic guanosine monophosphate in rats bearing Morris hepatoma 3924A. *Science* 190:59–60
85. Rabinowitz, B., Kligerman, M., Parmley, W. W. 1974. Plasma cyclic adenosine 3',5'-monophosphate (AMP) levels in acute myocardial infarction. *Am. J. Cardiol.* 34:7–11
86. Hamet, P., Kuchel, O., Fraysse, J., Genest, J. 1974. Plasma adenosine 3',5'-cyclic monophosphate in human hypertension. *Can. Med. Assoc. J.* 111:323–28
87. Grower, M. F., Ficara, A. J., Chandler, D. W., Kramer, G. D. 1975. Differences in cAMP levels in the gingival fluid of diabetics and nondiabetics. *J. Periodontol.* 46:669–72
88. Sproles, A. C. 1973. Cyclic AMP concentration in saliva of normal children and children with Down's syndrome. *J. Dent. Res.* 52:915–17
89. Weinryb, I., Chasin, M., Free, C. A., Harris, D. N., Goldenberg, H., Michel, I. M., Paik, V. S., Phillips, M., Samaniego, S., Hess, S. M. 1972. Effects of therapeutic agents on cyclic AMP metabolism in vitro. *J. Pharm. Sci.* 61:1556–67
90. Amer, M. S., McKinney, G. R. 1973. Possibilities for drug development based on the cyclic AMP system. *Life Sci.* 13:753–67
91. Breckenridge, B. M. 1970. Cyclic AMP and drug action. *Ann. Rev. Pharmacol.* 10:19–34
92. Weiss, B., ed. 1975. *Cyclic Nucleotides in Disease.* Baltimore: Univ. Park Press
93. Weiss, B., Fertel, R. 1977. *Adv. Pharmacol. Chemother.* 14:189–283
94. Uzunov, P., Shein, H. M., Weiss, B. 1974. Multiple forms of cyclic 3',5'-AMP phosphodiesterase of rat cerebrum and cloned astrocytoma and neuroblastoma cells. *Neuropharmacology* 13:377–91
95. Rall, T. W. 1969. In *The Role of Adenyl Cyclase and Cyclic 3',5'-AMP in Biological Systems, Fogerty Int. Proc., 4th, Natl. Inst. Health, Bethesda, Md.,* ed. T. W. Rall, M. Rodbell, P. Condliffe, pp. 7–15
96. Perkins, J. P. 1973. *Adv. Cyclic Nucleotide Res.* 3:1–64
97. Robison, G. A., Butcher, R. W., Sutherland, E. W. 1967. Adenyl cyclase as an adrenergic receptor. *Ann. NY Acad. Sci.* 139:703–23
98. Cheung, W. Y. 1970. Cyclic 3',5'-nucleotide phosphodiesterase. Demonstration of an activator. *Biochem. Biophys. Res. Commun.* 38:533–38
99. Brostrom, C. O., Huang, Y-C., Breckenridge, B. M., Wolff, D. J. 1975. Identification of a calcium-binding protein as a calcium-dependent regulator of brain adenylate cyclase. *Proc. Natl. Acad. Sci. USA* 72:64–68
100. Cheung, W. Y., Bradham, L. S., Lynch, T. J., Lin, Y. M., Tallant, E. A. 1975. Protein activator of cyclic 3':5'-nucleotide phosphodiesterase of bovine or rat brain also activates its adenylate cyclase. *Biochem. Biophys. Res. Commun.* 66:1055–62
101. Goldberg, N. D., O'Dea, R. F., Had-

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

dox, M. K. 1973. *Adv. Cyclic Nucleotide Res.* 3:155–224
102. Strada, S. J., Kirkegaard, L., Thompson, W. J. 1975. Studies of rat pineal gland guanylate cyclase. *Neuropharmacology* 15:261–66
103. Kimura, H., Murad, F. 1974. Evidence for 2 different forms of guanylate cyclase in rat heart. *J. Biol. Chem.* 249:6910–16
104. Chrisman, T. D., Garbers, D. L., Parks, M. A., Hardman, J. G. 1975. Characterization of particulate and soluble guanylate cyclases from rat lung. *J. Biol. Chem.* 250:374–81
105. Weiss, B., Costa, E. 1968. Selective stimulation of adenyl cyclase of rat pineal gland by pharmacologically-active catecholamines. *J. Pharmacol. Exp. Ther.* 161:310–19
106. George, W. J., Polson, J. B., O'Toole, A. G., Goldberg, N. D. 1970. Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc. Natl. Acad. Sci. USA* 66:398–403
107. Ferrendelli, J. A., Steiner, A. L., McDougal, D. B. Jr., Kipnis, D. M. 1970. The effect of oxotremorine and atropine on cGMP and cAMP levels in mouse cerebral cortex and cerebellum. *Biochem. Biophys. Res. Commun.* 41:1061–67
108. Thompson, W. J., Williams, R. H., Little, S. A. 1973. Activation of guanyl cyclase and adenyl cyclase by secretin. *Biochim. Biophys. Acta* 302:329–37
109. Rudland, P. S., Gospodarowicz, D., Seifert, W. 1974. Activation of guanyl cyclase and intracellular cyclic GMP by fibroblast growth factor. *Nature* 250:741–43
110. Stoner, J., Manganiello, V. C., Vaughan, M. 1974. Guanosine cyclic 3',5'-monophosphate and guanylate cyclase activity in guinea pig lung: Effects of acetylcholine and cholinesterase inhibitors. *Mol. Pharmacol.* 10:155–61
111. Ignarro, L. J., George, W. J. 1974. Mediation of immunologic discharge of lysosomal enzymes from human neutrophils by guanosine 3',5'-monophosphate. *J. Exp. Med.* 140:225–38
112. Appleman, M. M., Thompson, W. J., Russell, T. R. 1973. *Adv. Cyclic Nucleotide Res.* 3:65–98
113. Amer, M. S., Kreighbaum, W. E. 1975. Cyclic nucleotide phosphodiesterases: Properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. *J. Pharm. Sci.* 64:1–37
114. Weiss, B., Costa, E. 1968. Regional and subcellular distribution of adenyl cyclase and 3',5'-cyclic nucleotide phosphodiesterase in brain and pineal gland. *Biochem. Pharmacol.* 17:2107–16
115. Butcher, R. W., Sutherland, E. W. 1962. Adenosine 3',5'-phosphate in biological materials. I. Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine. *J. Biol. Chem.* 237:1244–50
116. Nair, K. G. 1966. Purification and properties of 3',5'-cyclic nucleotide phosphodiesterase from dog heart. *Biochemistry* 5:150–57
117. Campbell, M. T., Oliver, I. T. 1972. 3',5'-cyclic nucleotide phosphodiesterase in rat tissues. *Eur. J. Biochem.* 28:30–37
118. Beavo, J. A., Hardman, J. G., Sutherland, E. W. 1970. Hydrolysis of cyclic guanosine and adenosine 3',5'-monophosphates by rat and bovine tissues. *J. Biol. Chem.* 245:5649–55
119. Fertel, R., Weiss, B. 1974. A microassay for guanosine 3',5'-monophosphate phosphodiesterase activity. *Anal. Biochem.* 59:386–98
120. Fertel, R., Weiss, B. 1976. Properties and drug responsiveness of cyclic nucleotide phosphodiesterases of rat lung. *Mol. Pharmacol.* 12:678–87
121. Clark, J. F., Morris, H. P., Weber, G. 1973. Cyclic adenosine 3',5'-monophosphate phosphodiesterase activity in normal, differentiating, regenerating and neoplastic liver. *Cancer Res.* 33:356–61
122. Brooker, G., Thomas, L. J. Jr., Appleman, M. M. 1968. The assay of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate in biological materials by enzymatic radioisotopic displacement. *Biochemistry* 7:4177–81
123. Lagarde, A., Colobert, L. 1972. Cyclic 3',5'-AMP phosphodiesterase of human blood lymphocytes. *Biochem. Biophys. Acta* 276:444–53
124. Weiss, B., Strada, S. J. 1972. *Adv. Cyclic Nucleotide Res.* 1:357–74
125. Clark, R. B., Perkins, J. P. 1971. Regulation of adenosine 3',5'-cyclic monophosphate concentration in cultured human astrocytoma cells by catecholamines and histamine. *Proc. Natl. Acad. Sci. USA* 68:2757–60

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

126. Gilman, A. G., Nirenberg, M. 1971. Effect of catecholamines on the adenosine 3',5'-cyclic monophosphate concentration in clonal satellite cells of neurons. *Proc. Natl. Acad. Sci. USA* 68:2165–68
127. Strada, S. J., Klein, D. C., Weller, J., Weiss, B. 1972. Effect of norepinephrine on the concentration of adenosine 3',5'-monophosphate of rat pineal gland in organ culture. *Endocrinology* 90: 1470–75
128. Pan, P., Bonner, J. T., Wedner, H. J., Parker, C. W. 1974. Immunofluorescence evidence for the distribution of cyclic AMP in cells and cell masses of the cellular slime molds. *Proc. Natl. Acad. Sci. USA* 71:1623–25
129. Rosen, O. M. 1970. Interaction of cyclic GMP and cyclic AMP with a cyclic nucleotide phosphodiesterase of the frog erythrocyte. *Arch. Biochem. Biophys.* 139:447–49
130. Thompson, W. J., Appleman, M. M. 1971. Characterization of cyclic nucleotide phosphodiesterases of rat tissue. *J. Biol. Chem.* 246:3145–50
131. Song, S-Y., Cheung, W. Y. 1971. Cyclic 3',5'-nucleotide phosphodiesterase: properties of the enzyme of human blood platelets. *Biochim. Biophys. Acta* 242:593–605
132. Beavo, J. A., Hardman, J. G., Sutherland, E. W. 1971. Stimulation of adenosine 3',5'-monophosphate hydrolysis by guanosine 3',5'-monophosphate. *J. Biol. Chem.* 246:3841–46
133. Sung, C. P., Wiebelhaus, V. D., Jenkins, B. C., Adlercreutz, P., Hirschowitz, B. I., Sachs, G. 1972. Heterogeneity of 3',5'-phosphodiesterase of gastric mucosa. *Am. J. Physiol.* 223:648–50
134. Miller, J. P., Boswell, K. H., Muneyama, K., Simon, L. N., Robins, R. K., Shuman, D. A. 1973. Synthesis and biochemical studies of various 8-substituted derivatives of guanosine 3',5'-cyclic phosphate, inosine 3',5'-cyclic phosphate and xanthosine 3',5'-cyclic phosphate. *Biochemistry* 12: 5310–19
135. Amer, M. S., Mayol, R. F. 1973. Studies with phosphodiesterase III. Two forms of the enzyme from human blood platelets. *Biochim. Biophys. Acta* 309:149–56
136. Russell, T. R., Terasaki, W. L., Appleman, M. M. 1973. Separate phosphodiesterases for the hydrolysis of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate in rat liver. *J. Biol. Chem.* 248:1334–40
137. Brostrom, C. O., Wolff, D. J. 1976. Calcium-dependent cyclic nucleotide phosphodiesterase from brain: Comparison of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate as substrates. *Arch. Biochem. Biophys.* 172: 301–11
138. Russell, T. R., Thompson, W. J., Schneider, F. W., Appleman, M. M. 1972. 3',5'-cyclic adenosine monophosphate phosphodiesterase: Negative cooperativity. *Proc. Natl. Acad. Sci. USA* 69:1791–95
139. Loten, E. G., Sneyd, J. G. T. 1970. An effect of insulin on adipose tissue adenosine 3',5'-cyclic monophosphate phosphodiesterase. *Biochem. J.* 120:187–93
140. Weiss, B., Lehne, R., Strada, S. 1972. Rapid microassay of adenosine 3',5'-monophosphate phosphodiesterase activity. *Anal. Biochem.* 45:222–35
141. Schonhofer, P. S., Skidmore, I. F., Bourne, H. R., Krishna, G. 1972. Cyclic 3',5'-AMP phosphodiesterase in isolated fat cells. *Pharmacology* 7:65–77
142. Weiss, B., Strada, S. J. 1973. In *Fetal Pharmacology*, ed. L. Boreus, 205–32. New York: Raven
143. Strada, S. J., Uzunov, P., Weiss, B. 1974. Ontogenetic development of a phosphodiesterase activator and the multiple forms of cyclic AMP phosphodiesterase of rat brain. *J. Neurochem.* 23:1097–1103
144. Hait, W. N., Weiss, B. 1977. Isolation, characterization and selective inhibition of the multiple forms of phosphodiesterase of normal and leukemic lymphocytes. In preparation
145. Franks, D. J., MacManus, J. P. 1971. Cyclic GMP stimulation and inhibition of cyclic AMP phosphodiesterase from thymic lymphocytes. *Biochem. Biophys. Res. Commun.* 42:844–49
146. Thompson, W. J., Appleman, M. M. 1971. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry* 10:311–16
147. Goren, E. N., Hirsch, A. H., Rosen, O. M. 1971. Activity stain for the detection of cyclic nucleotide phosphodiesterase separated by polyacrylamide gel electrophoresis and its application to the cyclic nucleotide phosphodiesterase of beef heart. *Anal. Biochem.* 43:156–61
148. Monn, E., Christiansen, R. O. 1971. Adenosine 3',5'-monophosphate phosphodiesterase: Multiple molecular forms. *Science* 173:540–42
149. Uzunov, P., Weiss, B. 1972. Separation of multiple forms of cyclic adenosine

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

3',5'-monophosphate phosphodiesterase in rat cerebellum by polyacrylamide gel electrophoresis. *Biochim. Biophys. Acta* 284:220–26

150. Klotz, U., Berndt, S., Stock, K. 1972. Characterization of multiple cyclic nucleotide phosphodiesterase activities of rat adipose tissue. *Life Sci.* 11:7–17
151. Schroder, J., Rickenberg, H. V. 1973. Partial purification and properties of the cyclic AMP and the cyclic GMP phosphodiesterase of bovine liver. *Biochim. Biophys. Acta* 302:50–63
152. Pichard, A. L., Hanoune, J., Kaplan, J. C. 1973. Multiple forms of cyclic adenosine 3',5'-monophosphate phosphodiesterase from human blood platelets. *Biochem. Biophys. Acta* 315:370–77
153. Pledger, W. J., Stancel, G. M., Thompson, W. J., Strada, S. J. 1974. Separation of multiple forms of cyclic nucleotide phosphodiesterases from rat brain by isoelectrofocusing. *Biochim. Biophys. Acta* 370:242–48
154. Strada, S. J., Pledger, W. J. 1975. See Ref. 28, pp. 3–34
155. Wells, J. N., Baird, C. E., Wu, Y. J., Hardman, J. G. 1975. Cyclic nucleotide phosphodiesterase activities of pig coronary arteries. *Biochim. Biophys. Acta* 384:430–42
156. Singer, A. L., Sherwin, R. P., Dunn, A. S., Appleman, M. M. 1976. Cyclic nucleotide phosphodiesterases in neoplastic and nonneoplastic human mammary tissues. *Cancer Res.* 36:60–66
157. Uzunov, P., Shein, H. M., Weiss, B. 1973. Cyclic AMP phosphodiesterase in cloned astrocytoma cells: Norepinephrine induces a specific enzyme form. *Science* 180:304–6
158. Weiss, B., Fertel, R., Figlin, R., Uzunov, P. 1974. Selective alteration of the activity of the multiple forms of cyclic 3',5'-AMP phosphodiesterase of rat cerebrum. *Mol. Pharmacol.* 10:615–25
159. Weiss, B. 1975. *Adv. Cyclic Nucleotide Res.* 5:195–211
160. Wells, J. N., Wu, Y. J., Baird, C. E., Hardman, J. G. 1975. Phosphodiesterases from porcine coronary arteries: Inhibition of separated forms by xanthine, papaverine and cyclic nucleotides. *Mol. Pharmacol.* 11:775–83
161. Cheung, W. Y. 1971. Cyclic 3',5'-nucleotide phosphodiesterase evidence for and properties of a protein activator. *J. Biol. Chem.* 246:2859–69
162. Cheung, W. Y., Lin, Y. M., Liu, Y. P., Smoake, J. A. 1975. See Ref. 28, pp. 321–50
163. Kakiuchi, S., Yamazaki, R., Nakajima, H. 1970. Properties of a heat-stable phosphodiesterase activating factor isolated from brain extract. Studies on cyclic 3',5'-nucleotide phosphodiesterase II. *Proc. Jpn. Acad.* 46:587–92
164. Goren, E. N., Rosen, O. M. 1971. The effect of nucleotides and a non dialyzable factor on the hydrolysis of cyclic AMP by a cyclic nucleotide phosphodiesterase from beef heart. *Arch. Biochem. Biophys.* 142:720–23
165. Teo, T. S., Wang, J. H. 1973. Mechanism of activation of a cyclic adenosine 3',5'-monophosphate phosphodiesterase from bovine heart by calcium ions: Identification of the protein activator as a Ca^{2+} binding protein. *J. Biol. Chem.* 248:5950–55
166. Wolff, D. J., Brostrom, C. O. 1974. Calcium-binding phosphoprotein from pig brain: Identification as a calcium dependent regulator of rat brain nucleotide phosphodiesterase. *Arch. Biochem. Biophys.* 163:349–58
167. Wickson, R. D., Boudreau, R. J., Drummond, G. I. 1975. Activation of 3',5'-cyclic adenosine monophosphate phosphodiesterase by calcium ion and a protein activator. *Biochemistry* 14: 669–75
168. Levin, R. M., Weiss, B. 1976. Mechanism by which psychotropic drugs inhibit cyclic AMP phosphodiesterase of brain. *Mol. Pharmacol.* 12:581–89
169. Kakiuchi, S., Yamazaki, R. 1970. Calcium dependent phosphodiesterase activity and its activating factor (PAF) from brain. Studies on cyclic 3',5'-nucleotide phosphodiesterase (III). *Biochem. Biophys. Res. Commun.* 41: 1104–11
170. Wang, J. H., Teo, T. S., Ho, H. C., Stevens, F. C. 1975. *Adv. Cyclic Nucleotide Res.* 5:179–95
171. Lin, Y. M., Liu, Y. P., Cheung, W. Y. 1975. Cyclic 3',5'-nucleotide phosphodiesterase Ca^{2+}-dependent formation of bovine brain enzyme-activator complex. *FEBS Lett.* 49:356–60
172. Teshima, Y., Kakiuchi, S. 1974. Mechanism of stimulation of Ca^{2+} plus Mg^{2+}-dependent phosphodiesterase from rat cerebral cortex by the modulator protein and Ca^{2+}. *Biochem. Biophys. Res. Commun.* 56:489–95
173. Teo, T. S., Wang, J. H. 1973. Mechanism of activation of a cyclic adenosine 3',5'-monophosphate phosphodiesterase from bovine heart by calcium ions. *J. Biol. Chem.* 248:5950–55

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

174. Uzunov, P., Lehne, R., Revuelta, A. V., Gnegy, M. E., Costa, E. 1976. A kinetic analysis of the cyclic nucleotide phosphodiesterase regulation by the endogenous protein activator. A study of rat brain and frog sympathetic chain. *Biochim. Biophys. Acta* 422:326–34
175. Pledger, W. J., Thompson, W. J., Strada, S. J. 1975. Isolation of an activator of multiple forms of cyclic nucleotide phosphodiesterase of rat cerebrum by isoelectric focusing. *Biochim. Biophys. Acta* 391:334–40
176. Greenberg, L. H., Weiss, B. 1976. Activatable phosphodiesterase in various areas of rat brain. *Am. Soc. Neurochem.* 7:115
177. Uzunov, P., Revuelta, A., Costa, E. 1975. A role for the endogenous activator of 3'5';-nucleotide phosphodiesterase in rat adrenal medulla. *Mol. Pharmacol.* 11:506–10
178. Amer, M. S. 1975. Cyclic nucleotides in disease; On the biochemical etiology of hypertension. *Life Sci.* 17:1021–38
179. Ryan, W. L., Heidrick, M. L. 1974. *Adv. Cyclic Nucleotide Res.* 4:81–116
180. Johnson, G. S. 1975. See Ref. 28, pp. 35–44
181. Anderson, W. B., Russell, T. R., Carchman, R. A., Pastan, I. 1973. Interrelationship between adenylate cyclase activity, adenosine 3',5'-cyclic monophosphate phosphodiesterase activity, adenosine 3',5'-cyclic monophosphate levels, and growth of cells in culture. *Proc. Natl. Acad. Sci. USA* 70:3802–5
182. Rein, A., Carchman, R. A., Johnson, G. S., Pastan, I. 1973. Simian Virus 40 rapidly lowers cAMP levels in mouse cells. *Biochem. Biophys. Res. Commun.* 52:899–904
183. Hickie, R. A., Walker, C. M., Croll, G. A. 1974. Decreased basal cyclic adenosine 3',5'-monophosphate levels in Morris Hepatoma 5123 t.c.(h). *Biochem. Biophys. Res. Commun.* 59:167–73
184. Monahan, T. M., Marchand, N. W., Fritz, R. R., Abell, C. W. 1975. Cyclic adenosine 3',5'-monophosphate levels and activities of related enzymes in normal and leukemic lymphocytes. *Cancer Res.* 35:2540–47
185. Ryan, W. L., Heidrick, M. L. 1968. Inhibition of cell growth by adenosine 3',5'-monophosphate. *Science* 162: 1484–85
186. Gericke, D., Chandra, P. 1969. Inhibition of tumor growth by nucleoside cyclic 3',5'-monophosphates. *Hoppe-Seylers Z. Physiol. Chem.* 350:1469–71
187. Yang, T. J., Vas, S. I. 1971. Growth inhibitor effects of adenosine 3',5'-monophosphate on mouse leukemia L-5178-Y-R cells in culture. *Experientia* 27:442–44
188. Webb, D., Braun, W., Plescia, O. J. 1972. Antitumor effects of polynucleotides and theophylline. *Cancer Res.* 32:1814–19
189. Van Wijk, R., Wicks, W. D., Clay, K. 1972. Effects of derivatives of cyclic 3',5'-adenosine monophosphate on the growth, morphology, and gene expression of hepatoma cells in culture. *Cancer Res.* 32:1905–11
190. Keller, R., Keist, R. 1973. Suppression of growth of P-815 mastocytoma cells in vitro by drugs increasing cellular cyclic 3',5'-adenosine monophosphate. *Life Sci.* 12:97–105
191. Cho-Chung, Y. S., Gullino, P. M. 1974. In vivo inhibition of growth of two hormone-dependent mammary tumors by dibutyryl cyclic AMP. *Science* 183: 87–88
192. Hsie, A. W., Puck, T. T. 1971. Morphological transformation of Chinese hamster cells by dibutyryl adenosine cyclic 3',5'-monophosphate and testosterone. *Proc. Natl. Acad. Sci. USA* 68:358–61
193. Johnson, G. S., Friedman, R. M., Pastan, I. 1971. Restoration of several morphological characteristics of normal fibroblasts and sarcoma cells treated with adenosine-3',5'-cyclic monophosphate and its derivatives. *Proc. Natl. Acad. Sci. USA* 68:425–29
194. Prasad, K. M., Hsie, A. W. 1971. Morphologic differentiation of mouse neuroblastoma cells induced in vitro by dibutyryl adenosine 3',5' cyclic monophosphate. *Nature New Biol.* 233: 141–42
195. Ortiz, J., Yamada, T., Hsie, A. 1973. Induction of the stellate configuration in cultured iris epithelial cells by adenosine and compounds related to adenosine 3',5' cyclic monophosphate. *Proc. Natl. Acad. Sci. USA* 70:2286–90
196. Hsie, A., Kawashima, K., O'Neil, J. P., Schroder, C. H. 1975. Possible role of adenosine cyclic 3',5'-monophosphate phosphodiesterase in the morphological transformation of Chinese hamster ovary cells mediated by N^6,O^2-dibutyryl adenosine cyclic 3',5'-monophosphate. *J. Biol. Chem.* 250:984–89
197. Tisdale, M. J., Phillips, B. J. 1975. Inhibition of cyclic 3',5'-nucleotide phosphodiesterase—a possible mechanism of action of bifunctional alkylating

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

agents. *Biochem. Pharmacol.* 24:211–17
198. Tisdale, M. J. 1975. Characterisation of cyclic adenosine 3',5'-monophosphate phosphodiesterase from Walker carcinoma sensitive and resistant to bifunctional alkylating agents. *Biochim. Biophys. Acta* 397:134–43
199. Langan, T. A. 1969. Histone phosphorylation stimulation by adenosine 3',5'-monophosphate. *Science* 162:579–80
200. Bordy, S. C., Prasad, K. N., Purdy, J. L. 1974. Neuroblastoma: Drug induced differentiation increases proportion of cytoplasmic RNA that contains polyadenylic acid. *Science* 186:359–61
201. Fuhr, J. E., Overton, M., Yang, T. J. 1976. Stimulatory effect of 3',5'-adenosine monophosphate on protein synthesis in heat shocked murine leukemia lymphoblasts. *J. Natl. Cancer Inst.* 56:189–91
202. Willingham, M., Pastan, I. 1975. Cyclic AMP modulates microvillus formation and agglutinability in transformed and normal mouse fibroblasts. *Proc. Natl. Acad. Sci. USA* 72:1263–67
203. Tchao, R., Leighton, J. 1976. Inhibitory effect of dibutyryl cyclic AMP and theophylline on the aggregation of human breast tumor cell line BT-20. *Nature* 259:220–22
204. Voorhees, J. J., Duell, E. A., Bass, L. J., Powell, J. A., Harrell, E. R. 1972. Decreased cyclic AMP in the epidermis of lesions of psoriasis. *Arch. Dermatol.* 105:695–701
205. Voorhees, J. J., Stawiski, M., Ducll, E. A., Haddox, M. K., Goldberg, N. D. 1973. Increased cyclic GMP and decreased cyclic AMP levels in the hyperplastic abnormally differentiated epidermis of psoriasis. *Life Sci.* 13:639–53
206. Powell, J. A., Duell, E. A., Voorhees, J. J. 1971. Beta adrenergic stimulation of endogenous epidermal cyclic AMP formation. *Arch. Dermatol.* 104:359–65
207. Wright, R. K., Mandy, S. H., Halprin, K. M., Hsia, S. L. 1973. Defects and deficiency of adenyl cyclase in psoriatic skin. *Arch. Dermatol.* 107:47–53
208. Voorhees, J. J., Duell, E. A., Stawiski, M., Creehan, P., Harrell, E. R. 1975. See Ref. 28, pp. 79–101
209. Stawiski, M. A., Powell, J. A., Lang, F. G., Schork, M. A., Duell, E. A., Voorhees, J. J. 1975. Papaverine: Its effects on cyclic AMP in vitro and psoriasis in vivo. *J. Invest. Dermatol.* 64:124–27
210. Szentivanyi, A. 1968. The beta adrenergic theory of the atopic abnormality in bronchial asthma. *J. Allergy* 42:203–32
211. Cookson, D. U., Reed, C. E. 1963. A comparison of the effects of isoproterenol in the normal and asthmatic subject. *Am. Rev. Respir. Dis.* 88: 636–43
212. Kirkpatrick, C. H., Keller, C. 1967. Impaired responsiveness to epinephrine in asthma. *Am. Rev. Respir. Dis.* 96: 692–99
213. Parker, C. W., Smith, J. W. 1973. Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. *J. Clin. Invest.* 52:48–59
214. Taylor, W. A., Francis, D. H., Sheldon, D., Roitt, I. M. 1974. Anti-allergic actions of disodium cromoglycate and other drugs known to inhibit cyclic 3',5'-nucleotide phosphodiesterase. *Int. Arch. Allergy Appl. Immunol.* 47: 175–93
215. Tateson, J. E., Trist, D. G. 1976. Inhibition of adenosine 3',5'-cyclic monophosphate phosphodiesterase by potential antiallergic compounds. *Life Sci.* 18:153–62
216. Davies, G. E., Evans, D. P. 1973. Studies with two new phosphodiesterase inhibitors (ICI 58, 301 and ICI 63, 197) on anaphylaxis in guinea pigs, mice and rats. *Int. Arch. Allergy Appl. Immunol.* 45:467–78
217. Weiss, B., Davies, J. I., Brodie, B. B. 1966. Evidence for a role of adenosine 3',5'-monophosphate in adipose tissue lipolysis. *Biochem. Pharmacol.* 15: 1553–61
218. Lichtenstein, L. M., Margolis, S. 1968. Histamine release in vitro: Inhibition by catecholamines and methylxanthines. *Science* 161:902–3
219. Ishizuka, M., Braun, W., Matsumoto, T. 1971. Cyclic AMP and immune responses: I. Influence of poly A:U and cAMP on antibody formation in vitro. *J. Immunol.* 107:1027–35
220. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L. 1972. Pharmacologic control of allergic histamine release in vitro: Evidence for an inhibitory role of 3',5'-adenosine monophosphate in human leukocytes. *J. Immunol.* 108:695–705
221. Gillespie, E. 1973. Compound 48/80 decreases adenosine 3',5'-monophosphate formation in rat peritoneal mast cells. *Experientia* 29:447–48
222. Sullivan, T., Parker, C. W. 1973. Cyclic AMP phosphodiesterase activation by the histamine releasing agent, compound 48/80. *Biochem. Biophys. Res. Commun.* 55:1334–39

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

223. Roy, A. C., Warren, B. T. 1974. Inhibition of cAMP phosphodiesterase by disodium cromoglycate. *Biochem. Pharmacol.* 23:917–20
224. Lavin, N., Rachelefsky, G. S., Kaplan, S. A. 1975. An action of disodium cromoglycate: Inhibition of cyclic 3',5'-AMP phosphodiesterase. *J. Allergy Clin. Immunol.* 57:80–88
225. Brodie, B. B., Davies, J. I., Hynie, S., Krishna, G., Weiss, B. 1966. Interrelationships of catecholamines with other endocrine systems. *Pharmacol. Rev.* 18:273–89
226. Lavin, N., Rachelefsky, G., Kaplan, S. A. 1975. Inhibition of cyclic AMP phosphodiesterase in human lymphocytes by physiological concentrations of hydrocortisone. *Horm. Metab. Res.* 7:253–58
227. Manganiello, V., Vaughan, M. 1972. An effect of dexamethasone on adenosine 3',5'-monophosphate content and adenosine 3',5'-monophosphate phosphodiesterase activity of cultured hepatoma cells. *J. Clin. Invest.* 51:2763–67
228. Weissman, G., Zurier, R. B., Hoffstein, S. 1974. See Ref. 14, pp. 176–88
229. Lichtenstein, L. M., 1974. See Ref. 14, pp. 142–62
230. Stefanovich, V. 1974. Inhibition of 3',5'-cyclic AMP phosphodiesterase with anti-inflammatory agents. *Res. Commun. Chem. Pathol. Pharmacol.* 7:573–82
231. Newcombe, D. S., Thanassi, N. M., Ciosek, C. P. Jr. 1974. Cartilage cyclic nucleotide phosphodiesterase: Inhibition by anti-inflammatory agents. *Life Sci.* 14:505–19
232. Kupiecki, F. P., Adams, L. D. 1974. The lipolytic system in adipose tissue of Toronto-KK and C57BL/K_sJ diabetic mice. Adenylate cyclase, phosphodiesterase and protein kinase activities. *Diabetologia* 10:633–37
233. Kukovetz, W. R., Poch, G. 1970. Inhibition of cyclic 3',5'-nucleotide phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs. *Arch. Pharmakol.* 267:189–94
234. Markwardt, F., Hoffmann, A. 1970. Effects of papaverine derivatives on cyclic AMP phosphodiesterase of human platelets. *Biochem. Pharmacol.* 19: 2519–20
235. Beavo, J. A., Rogers, N. L., Crofford, O. B., Hardman, J. G., Sutherland, E. W., Newman, E. V. 1970. Effects of xanthine derivatives on lipolysis and adenosine 3',5'-monophosphate phosphodiesterase activity. *Mol. Pharmacol.* 6:597–603
236. Schultz, G., Senft, G., Losert, W., Sitt, R. 1966. Biochemische grundlagen der diazoxidhyperglykamie. *Naunyn Schmiedeberg's Arch. Exp. Pathol. Pharmacol.* 253:372–87
237. Moore, P. F. 1968. The effects of diazoxide and benzothiadiazine diuretics upon phosphodiesterase. *Ann. NY Acad. Sci.* 150:256–60
238. Nitz, R. E., Schraven, E., Trottnow, D. 1968. Hemmung der Phosphodiesterase aus Rattenherzen durch Intensain. *Experientia* 24:334–35
239. Orloff, J., Handler, J. S. 1962. The similarity of effects of vasopressin, adenosine 3',5'-phosphate (cyclic AMP) and theophylline on the toad bladder. *J. Clin. Invest.* 41:702–9
240. Gadd, R. E. A., Clayman, S., Herbert, D. 1973. Inhibition of cyclic 3',5'-nucleotide phosphodiesterase activity by diuretics and other agents. *Experientia* 29:1217–19
241. Senft, G., Schultz, G., Munske, K., Hoffmann, M. 1968. Effects of glucocorticoids and insulin on 3',5'-AMP phosphodiesterase activity in adrenalectomized rats. *Diabetologia* 4:330–35
242. Levey, G. S. 1975. See Ref. 28, pp. 157–64
243. Sobel, B. E., Henry, P. D., Robison, A., Bloor, C., Ross, J. Jr. 1969. Depressed adenyl cyclase activity in the failing guinea pig heart. *Circ. Res.* 24:507–12
244. Gold, H. K., Prindle, K. H., Levey, G. S., Epstein, S. E. 1970. Effects of experimental heart failure on the capacity of glucagon to augment myocardial contractility and activate adenyl cyclase. *J. Clin. Invest.* 49:999–1006
245. Sulakhe, P. V., Dhalla, N. S. 1972. Adenyl cyclase activity in failing hearts of genetically myopathic hamsters. *Biochem. Med.* 6:471–82
246. Lippmann, W. 1974. Inhibition of beef heart and rat brain nucleoside-3',5'-monophosphate phosphodiesterase by 3 β-14-dihydroxy-21-oxo-23-desoxo-5-β-card-20(22)-enolide 3 acetate (AY-17,605) and structurally related compounds. *Experientia* 30:237–39
247. Shimamoto, T. 1975. Hyperactive arterial endothelial cells in atherogenesis and cyclic AMP phosphodiesterase inhibitor in prevention and treatment of atherosclerotic disorders. *Jpn. Heart J.* 16:76–97
248. Iwai, H. 1974. Inhibition of protein kinase and cyclic AMP phosphodiester-

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

ase by eritadenine isoamyl ester. *J. Biochem.* 76:419–29
249. Goldfine, I. D., Perlman, R., Roth, J. 1971. Inhibition of cyclic 3',5'-AMP phosphodiesterase in islet cells and other tissues by tolbutamide. *Nature* 234:295–96
250. Rosen, O., Hirsch, A., Goren, E. N. 1971. Factors which influence cyclic AMP formation and degradation in an islet cell tumor of the Syrian hamster. *Arch. Biochem. Biophys.* 146:660–63
251. Ashcroft, S. J. H., Randle, P. J., Taljedal, I. B. 1972. Cyclic nucleotide phosphodiesterase in normal mouse pancreatic islets. *FEBS Lett.* 20:263–65
252. Webb, D. R., Bourne, H. R., Levinson, W. 1974. New phosphodiesterase inhibitors in human lymphocytes N-methylisatin-beta-thiosemicarbazone. *Biochem. Pharmacol.* 23:1663–67
253. Uzunov, P., Weiss, B. 1971. Effects of phenothiazine tranquilizers on the cyclic 3',5'-adenosine monophosphate system of rat brain. *Neuropharmacology* 10:697–708
254. Kebabian, J. W., Petzold, G. L., Greengard, P. 1972. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor." *Proc. Natl. Acad. Sci. USA* 69:2145–49
255. Palmer, G. C., Jones, D. J., Medina, M. A., Stavinoha, W. B. 1975. Action of psychoactive drugs on cyclic AMP levels in mouse crebral cortex and lung following microwave irradiation. *Pharmacologist* 17:233
256. Beer, B., Chasin, M., Clody, D. E., Vogel, J. R., Horovitz, Z. P. 1972. Cyclic adenosine monophosphate phosphodiesterase in brain: Effect on anxiety. *Science* 176:428–30
257. Dalton, C., Crowley, H. J., Sheppard, H., Schallek, W. 1974. Regional cyclic nucleotide phosphodiesterase activity in cat central nervous system: Effects of benzodiazepines (37820). *Proc. Soc. Exp. Biol. Med.* 145:407–10
258. Berndt, S., Schwabe, U. 1973. Effect of psychotropic drugs on phosphodiesterase and cyclic AMP level in rat brain in vivo. *Brain Res.* 63:303–12
259. Pichard, A. L., Hanoune, J., Kaplan, J. C. 1972. Human brain and platelet cyclic adenosine 3',5'-monophosphate phosphodiesterases: Different response to drugs. *Biochim. Biophys. Acta* 279:217–20
260. Muschek, L. D., McNeill, J. H. 1971. The effect of tricyclic antidepressants and promethazine on 3',5'-cyclic AMP phosphodiesterase from rat brain. *Fed. Proc.* 30:330
261. Vernikos-Danellis, J., Harris, C. G. III. 1968. The effect of in vitro and in vivo caffeine, theophylline and hydrocortisone on the phosphodiesterase activity of the pituitary, median eminence, heart, and cerebral cortex of the rat. *Proc. Soc. Exp. Biol. Med.* 128:1016–21
262. Chasin, M., Harris, D. N., Phillips, M. B., Hess, S. M. 1972. 1-Ethyl-4-(isopropylidenehydrazino)-1H-pyrazolo-(3, 4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride (SQ 20,009)—A potent new inhibitor of cyclic 3',5'-nucleotide phosphodiesterases. *Biochem. Pharmacol.* 21:2443–50
263. Palmer, G. C., Robison, G. A., Sulser, F. 1971. Modification by psychotropic drugs of the cyclic adenosine monophosphate response to norepinephrine in rat brain. *Biochem. Pharmacol.* 20:236–39
264. Monahan, T. M., Fritz, R. R., Abell, C. W. 1973. Levels of cyclic AMP in murine L5178Y lymphoblasts grown in different concentrations of serum. *Biochem. Biophys. Res. Commun.* 11: 642–46
265. Coffey, R. G., Logsdon, P. S., Middleton, E. 1973. Leucocyte adenyl cyclase and ATPase in asthma: Effect of corticosteroid therapy. *Chest* 63:25–35
266. Alston, W. C., Patch, K. R., Kerr, J. W. 1974. Response of leucocyte adenyl cyclase to isoprenaline and effects of alpha blocking drugs in extrinsic bronchial asthma. *Br. Med. J.* 1:90–93
267. Gillespie, E., Valentine, M. D., Lichtenstein, L. M. 1974. Cyclic AMP metabolism in asthma: Studies with leucocytes and lymphocytes. *J. Allergy Clin. Immunol.* 53:27–33
268. Fireman, P., Palm, C. R., Friday, G. A. 1970. Metabolic responses to epinephrine in asthmatic, eczematous and normal subjects. *J. Allergy* 45:117
269. Sharma, R. K. 1972. Studies on adrenocortical carcinoma of rat cyclic nucleotide phosphodiesterase activities. *Cancer Res.* 32:1734–36
270. Thomas, E. W., Murad, F., Looney, W. B., Morris, H. P. 1973. Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate: Concentrations in Morris hepatomas of different growth rates. *Biochim. Biophys. Acta* 297: 564–67

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

271. Boyd, H., Louis, C. J., Martin, T. J. 1974. Activity and hormone responsiveness of adenyl cyclase during induction of tumors in rat liver with 3'-methyl-4-dimethylaminoazobenzene *Cancer Res.* 34:1720–25
272. Chayoth, R., Epstein, S. M., Field, J. B. 1973. Glucagon and prostaglandin E_1 stimulation of cyclic adenosine 3',5'-monophosphate levels and adenylate cyclase activity in benign hyperplastic nodules and malignant hepatomas of ethionine treated rates. *Cancer Res.* 33:1970–74
273. Chayoth, R., Epstein, S. M., Field, J. B. 1972. Increased cyclic AMP levels in malignant hepatic nodules of ethionine treated rats. *Biochem. Biophys. Res. Commun.* 49:1663–70
274. Clark, J. F., Morris, H. P., Weber, G. 1973. Cyclic adenosine 3',5'-monophosphate phosphodiesterase activity in normal, differentiating, regenerating and neoplastic liver. *Cancer Res.* 33:356–61
275. Makman, M. H. 1971. Conditions leading to enhanced response to glucagon, epinephrine, or prostaglandins by adenylate cyclase of normal and malignant cultured cells. *Proc. Natl. Acad. Sci. USA* 68:2127–30
276. Hickie, R. A., Walker, C. M., Datta, A. 1975. Increased activity of low-Km cyclic adenosine 3',5'-monophosphate phosphodiesterase in plasma membranes of Morris hepatoma 5123tc. *Cancer Res.* 35:601–5
277. Kimura, H., Murad, F. 1975. Increased particulate and decreased soluble guanylate cyclase activity in regenerating liver, fetal liver and hepatoma. *Proc. Natl. Acad. Sci. USA* 72:1965–69
278. Kemp, R. G., Duquesnoy, R. J. 1974. Thymus adenylate cyclase activity during murine leukemogenesis. *Science* 18:218–19
279. Konings, A. W. T., Pierce, D. A. 1974. Hydrolysis of 2'3'-cAMP and 3',5' cAMP in subcellular fractions of normal and neoplastic mouse spleen. *Life Sci.* 15:491–99
280. Perkins, J. P., Macintyre, E. H., Riley, W. D., Clark, R. B. 1971. Adenyl cyclase, phosphodiesterase and cyclic AMP dependent protein kinase of malignant glial cells in culture. *Life Sci.* 10:1069–80
281. Cohen, L., Chan, P-C. 1974. Intracellular cAMP levels in normal rat mammary gland and adenocarcinoma in vivo vs. in vitro. *Life Sci.* 16:107–15
282. Chatterjee, S. K., Kim, U. 1976. Biochemical properties of cyclic nucleotide phosphodiesterase in metastasizing and non-metastasizing rat mammary carcinomas. *J. Natl. Cancer Inst.* 56: 105–10
283. Brown, H. D., Chattopadhyay, S. K., Spjut, H. J., Spratt, J. S. Jr., Pennington, S. N. 1969. Adenyl cyclase activity in dimethylamino biphenyl-induced breast carcinoma. *Biochim. Biophys. Acta* 192:372–75
284. Minton, J. P., Wisenbaugh, T., Matthews, R. H. 1974. Elevated cyclic AMP levels in human breast cancer tissue. *J. Natl. Cancer Inst.* 53:283–84
285. Raymond, M. K., Schmitt, G., Galsky, A. G. 1975. Cyclic AMP phosphodiesterase in crown gall tumor formation. *Biochem. Biophys. Res. Commun.* 66:222–26
286. Verma, A. K., Murray, A. W. 1974. The effect of benzo(a)pyrene on the basal and isoproterenol stimulated levels of cyclic adenosine 3',5'-monophosphate in mouse epidermis. *Cancer Res.* 34:3408–13
287. Lincoln, T., Vaughan, G. L. 1975. The role of adenosine 3',5'-monophosphate in the transformation of Cloudman mouse melanoma cells. *J. Cell. Physiol.* 86:543–52
288. Belman, S., Troll, W. 1974. Phorbol-12-myristate-13-acetate effect on cyclic adenosine 3',5'-monophosphate levels in mouse skin and inhibition of phorbol myristate acetate promoted tumor genesis. *Cancer Res.* 34:3446–55
289. Verma, A. K., Froscio, M., Murray, A. 1976. Croton oil-and benzo(a) pyrene-induced changes in cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate phosphodiesterase activities in mouse epidermis. *Cancer Res.* 36:81–87
290. Anderson, W. B., Johnson, G. S., Pastan, I. 1973. Transformation of chick-embryo fibroblasts by wild-type and temperature-sensitive Rous sarcoma virus alters adenylate cyclase activity. *Proc. Natl. Acad. Sci. USA* 70:1055–59
291. Otten, J., Bader, J., Johnson, G. S., Pastan, I. 1972. A mutation in a Rous sarcoma virus gene that controls adenosine 3',5'-monophosphate levels and transformation. *J. Biol. Chem.* 247:1632–33
292. Otten, J., Johnson, G. S., Pastan, I. 1972. Regulation of cell growth by cyclic adenosine 3',5'-monophosphate. Effect of cell density and agents which alter cell growth on cyclic adenosine

Annu. Rev. Pharmacol. Toxicol. 1977.17:441-477. Downloaded from www.annualreviews.org by Central College on 12/14/11. For personal use only.

3',5'-monophosphate levels in fibroblasts. *J. Biol. Chem.* 247:7082–87
293. Lynch, T. J., Tallant, E. A., Cheung, W. Y. 1975. Marked reduction of cyclic GMP phosphodiesterase activity in virally transformed mouse fibroblasts. *Biochem. Biophys. Res. Commun.* 65:1115–22
294. Peery, C. V., Johnson, G. S., Pastan, I. 1971. Adenyl cyclase in normal and transformed fibroblasts in tissue culture: Activation by prostaglandins. *J. Biol. Chem.* 246:5785–90
295. Augustyn, J. M., Ziegler, F. D. 1975. Endogenous cyclic adenosine monophosphate in tissues of rabbits fed an atherogenic diet. *Science* 187:449–50
296. Goldstein, R. E., Skelton, C. L., Levy, G. S., Glancy, D. L., Beiser, G. D., Epstein, S. E. 1971. Effects of chronic heart failure on the capacity of glucagon to enhance contractility and adenyl cyclase activity of human papillary muscles. *Circulation* 44:638–47
297. Ling, W. Y., Marsh, J. M., Spellacy, W. N., Thresher, A. J., Lemaire, W. J. 1974. Adenosine 3',5'-monophosphate in amniotic fluid from pregnancy complicated by hypertension. *J. Clin. Endocrinol. Metab.* 39:479–86
298. Klenerova, V., Albrecht, I., Hynie, S. 1975. The activity of adenylate cyclase and phosphodiesterase in hearts and aortas of spontaneous hypertensive rats. *Pharmacol. Res. Commun.* 7:453–62
299. Ramanathan, S., Shibata, S. 1974. Cyclic AMP in blood vessels of the spontaneously hypertensive rat. *Blood Vessels* 11:312–18
300. Ramanathan, S., Shibata, S., Tasaki, T. K., Ichord, R. N. 1976. Cyclic AMP metabolism in the cardiac tissue of the spontaneously hypertensive rat. *Biochem. Pharmacol.* 25:223–25
301. Hamet, P., Kuchel, O., Genest, J. 1973. Effect of upright posture and isoproterenol infusion on cyclic adenosine monophosphate excretion in control subjects and patients with labile hypertension. *J. Clin. Endocrinol. Metab.* 36:218–26
302. Kaminsky, N. I., Broadus, A. E., Hardman, J. G., Jones, D. J., Ball, J. H., Sutherland, E. W., Liddle, G. W. 1970. Effects of parathyroid hormone on plasma and urinary adenosine 3',5'-monophosphate in man. *J. Clin. Invest.* 49:2387–95
303. Taylor, A. L., Davis, B. B., Pawlson, L. G., Josinovich, J. B., Mintz, D. H. 1970. Factors influencing the urinary excretion of 3',5'-adenosine monophosphate in humans. *J. Clin. Endocrinol. Metab.* 30:316–24
304. Rosen, O. M. 1972. Urinary cyclic AMP in Grave's disease. *N. Engl. J. Med.* 287:670–71
305. Grill, V., Rosenqvist, U. 1975. Accumulation of cyclic AMP in hypothyroidism. Decreased sensitivity to norepinephrine in rat adipocytes. *Acta Endocrinol.* 78:39–43
306. Farber, D. B., Lolley, R. N. 1974. Cyclic guanosine monophosphate: Elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science* 186:449–51
307. Myllyla, V. V., Heikkinen, E. R., Vapaatalo, H., Hokkanen, E. 1975. Cyclic AMP concentration and enzyme activities of cerebrospinal fluid in patients with epilepsy or central nervous system damage. *Eur. Neurol.* 13:123–39
308. Sattin, A. 1971. Increase in the content of adenosine 3',5'-monophosphate in mouse forebrain during seizures and prevention of the increase by methylxanthines. *J. Neurochem.* 18:1087–96
309. Skolnick, P., Daly, J. W. 1974. The accumulation of adenosine 3',5'-monophosphate in cerebral cortical slices of the quaking mouse, a neurologic mutant. *Brain Res.* 73:513–25