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DIFFERENTIAL EFFECTS OF CYCLIC NUCLEOTIDES AND THEIR ANALOGS AND VARIOUS AGENTS ON CYCLIC GMP-SPECIFIC AND CYCLIC AMP-SPECIFIC PHOSPHODIESTERASES PURIFIED FROM GUINEA PIG LUNG*

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Abstract—The differential effects of various compounds on the activities of cyclic GMP-specific phosphodiesterase (cyclic GMP-PDE) and cyclic AMP-specific phosphodiesterase (cyclic AMP-PDE) purified from the guinea pig lung were examined. Cyclic IMP, 2'-deoxy cyclic GMP and 2'-*O*-monobutyryl cyclic GMP were found to be the most potent inhibitors of the cyclic GMP-PDE, with I_{50} (concentrations of the compounds inhibiting 50 per cent of the activity) values of 1.5, 4.5 and 35 μM respectively. These compounds, however, were at least 30-600 times less potent in inhibiting the cyclic AMP-PDE. In contrast, 2'-*O*-monobutyrul cyclic AMP and 2'-deoxy cyclic AMP were the most potent inhibitors of cyclic AMP-PDE, with I_{50} values of 10 and 45 μM respectively; these compounds, however, were at least 30-100 times less potent in inhibiting cyclic GMP-PDE. Ethanol (13%, v/v) stimulated cyclic GMP-PDE 80 per cent while conversely inhibiting cyclic AMP-PDE 75 per cent. Most of the phosphodiesterase inhibitors studied were found to be more selective for cyclic AMP-PDE, with the possible exception of 1-methyl-3-isobutylxanthine, which was more specific for inhibiting cyclic GMP-PDE. The differential inhibition of the two classes of phosphodiesterases by a wide variety of compounds shown in the present study suggests a possibility of selective regulation of the tissue levels of respective cyclic nucleotides through specific or preferential inhibition of their enzyme activities.

The biological actions of cyclic GMP and cyclic AMP appear to be unique, and in some instances, mutually opposing [1, 2]. Alterations of the cyclic nucleotide systems have been associated with a variety of pathophysiological states [3-9], suggesting a prominent role for these agents in these processes. Agents which could selectively alter the intracellular levels of either cyclic GMP or cyclic AMP may prove beneficial in correcting these disorders. Multiple forms of cyclic nucleotide phosphodiesterases (EC 3.1.4 C) have been shown to exist in a wide variety of tissues [10-15] and, in some cases, enzymes with a relative specificity for hydrolyzing either cyclic GMP or cyclic AMP have been observed [16-24]. Differential inhibition of enzymes from different tissues [25-31], as well as of enzymes from the same tissue [18, 29, 32, 33] by various agents, suggests that selective changes in cyclic nucleotide levels may be possible through alteration in the activity of specific phosphodiesterases.

We have previously reported [34] that guinea pig lung contains a low K_m form of phosphodiesterase activity specific for cyclic GMP and an activity specific for cyclic AMP, which could account for all the cyclic GMP and cyclic AMP hydrolysis by tissue extract. More recently, the pulmonary cyclic GMP-specific phosphodiesterase (cyclic GMP-PDE) has been purified to about 50 per cent homogeneity and its properties have been extensively characterized [35]. The present investigation describes the effects of several cyclic nucleotides and their analogs, as well as a wide variety of compounds, on the activities of cyclic GMP-PDE, and cyclic AMP-specific phosphodiesterase (cyclic AMP-PDE) purified from the same tissue. The differential effects of these agents on the two classes of enzymes indicate that selective alteration of cyclic nucleotide levels in the lung, through alteration in the activities of phosphodiesterase, seems to be possible.

EXPERIMENTAL PROCEDURE

Materials. Cyclic [G-^{3}H]GMP (9.8 Ci/m-mole) and cyclic [G-^{3}H]AMP (27.9 Ci/m-mole) were purchased from New England Nuclear; AGl-X8 (Cl$^-$ form, 100-200 mesh), was from Bio-Rad; and snake venom (*Crotalus adamanteus*) was from Sigma. Various cyclic nucleotides and their analogs were purchased from either Sigma, Boehringer Mannheim or Plenum Scientific Research. Caffeine, theophylline, and 1-methyl-3-isobutylxanthine were from Aldrich Chemi-

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cals. Ro 7-2956 and Ro 20-1724 were the generous gifts of Dr. Herbert Sheppard (Hoffman-LaRoche). Sch 15280 was a gift from Dr. William Kreutner (Schering) and SQ 20,009 was from Squibb.

Methods. All experiments were performed using mature female guinea pigs (600–800 g). Animals were killed by decapitation and the tissues were immediately excised and rinsed in ice-cold 50 mM Tris–Cl buffer (pH 7.5) containing 3.75 mM 2-mercaptoethanol (extraction buffer). The tissues were homogenized in 2 vol. of extraction buffer for 10 sec using either a Sorvall Omnimixer (maximal speed) or Brinkmann Polytron (setting 6). The homogenate was centrifuged at 105,000 *g* for 45 min and the supernatant (crude extract) was filtered through glass wool.

The pulmonary cyclic GMP–PDE and cyclic AMP–PDE were purified as described elsewhere [34, 35]. Briefly, cyclic GMP–PDE was obtained following the steps of DEAE–cellulose chromatography, hydroxylapatite gel treatment and preparatory acrylamide gel electrophoresis [35]. The specific activity (units/mg of protein) of the resulting enzyme was 27,000, which represented a 250–fold purification over the activity present in the crude extract. Analytical gel electrophoresis revealed a doublet protein band, indicating the enzyme preparation was at least 50 per cent homogenous. The relative rate of hydrolysis of cyclic GMP compared to that of cyclic AMP, using 1 μM substrate concentrations, was at least 1000 to 1. The cyclic AMP–PDE was purified 7-fold using DEAE–cellulose [34, 35] and hydrolyzed cyclic AMP 7–15 times more readily than cyclic GMP using 1 μM substrate concentrations.

The assay method for phosphodiesterase was a modification [34] of that described by Thompson and Appleman [36]. In brief, the standard reaction mixture contained, in a final volume of 0.1 ml, Tris–Cl buffer, pH 8.0, 5 μmoles; $MgCl_2$, 4 μmoles; 5′-nucleotidase (*C. adamanteus* snake venom), 10–30 μg; cyclic [G-^{3}H]AMP, 0.1 nmole, containing about 1 × 10^5 cpm. The reaction was initiated by addition of either the substrate or the enzyme and was carried out at 37°. The reaction was terminated by heating the reaction mixture at 98–100° for 1 min. The unreacted nucleotides were separated from dephosphorylated products using anion exchange chromatography. Appropriate amounts (0.04 to 20 μg) of the enzymes and appropriate incubation times (5–20 min) were employed so that 10–30 per cent of the cyclic nucleotides was hydrolyzed under the assay conditions. Linear reaction kinetics were observed under the assay conditions. None of the compounds examined were shown to affect the conversion of 5′-nucleotides, by 5′-nucleotidease in the snake venom, to their corresponding nucleosides. One unit of enzyme activity is defined as that amount of activity that hydrolyzes 1 pmole of cyclic nucleotide/min under the assay conditions. All assays were performed in duplicate and the values are corrected for those obtained in the absence of added phosphodiesterase. Cyclic nucleotides and cyclic nucleotide analogs were dissolved in water, and other compounds were dissolved in 10% dimethyl sulfoxide, which did not significantly alter enzyme activities. Kinetic analyses were performed using double reciprocal plots with the slope and intercept determined by linear least square analysis. The amounts of protein were determined by the method of Lowry *et al.* [37], using bovine serum albumin as a standard.

Inhibition of phosphodiesterase activities by cyclic nucleotides and their analogs. 2′-*O*-monobutyryl cyclic GMP was more effective than 2′-*O*-monobutyryl cyclic AMP in inhibiting cyclic GMP–PDE, while the converse was found for inhibition of cyclic AMP–PDE (Fig. 1). While N^2-monobutyryl cyclic GMP was more effective than N^6-monobutyryl cyclic AMP in inhibiting cyclic GMP–PDE, they were nearly equally potent in inhibiting cyclic AMP–PDE. The dibutyryl derivatives of either cyclic nucleotide were equally potent inhibitors of both enzymes.

Table 1 summarizes the effect of these and some other compounds on the hydrolysis of cyclic GMP or cyclic AMP by their respective phosphodiesterases purified from guinea pig lung. Cyclic IMP and 2′-deoxy cyclic GMP were the most potent inhibitors of the cyclic GMP–PDE, with I_{50} values of 1.5 and 4.5 μM respectively. In contrast to cyclic IMP, the 8-bromo and 8-benzylamino derivatives of cyclic IMP were very weak inhibitors of the cyclic GMP–enzyme. Of the cyclic AMP derivatives, 8-benzylamino cyclic

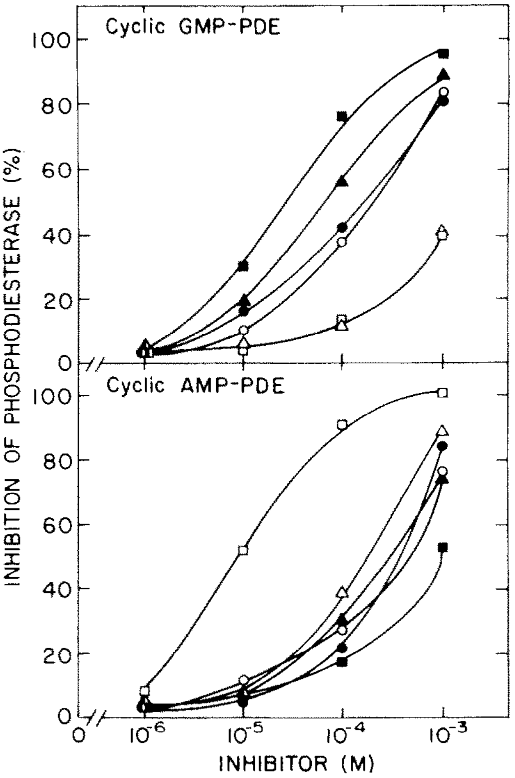

Fig. 1. Inhibition by cyclic nucleotide analogs of cyclic GMP– and cyclic AMP–PDE purified from guinea pig lungs. Cyclic GMP–PDE (0.04 μg) and cyclic AMP–PDE (4 μg) were from the gel electrophoresis [35] and DEAE–cellulose [34, 35] steps respectively. The activities of the respective enzymes for hydrolyzing 1 μM cyclic GMP and cyclic AMP were 1.15 and 3.29 units. Key: dibutyryl cyclic GMP (●); N^2-monobutyryl cyclic GMP (▲); 2′-*O*-monobutyryl cyclic GMP (■); dibutyryl cyclic AMP (○); N^6-monobutyryl cyclic AMP (△); and 2′-*O*-monobutyryl cyclic AMP (□).

Table 1. Summary of I_{50} values for various cyclic nucleotides and their derivatives on cyclic GMP–PDE or cyclic AMP–PDE purified from guinea pig lung*

Cyclic nucleotide or derivative	I_{50} (μM) Cyclic GMP–PDE	I_{50} (μM) Cyclic AMP–PDE
Cyclic IMP	1.5	> 1000
2′-Deoxy cyclic GMP	4.5	> 1000
2′-*O*-Monobutyryl cyclic GMP	35	900
8-Benzylamino cyclic AMP	78	350
N^2-monobutyryl cyclic GMP	80	280
N^2,2′-*O*-dibutyryl cyclic GMP	150	460
8-Methylthio cyclic AMP	170	110
N^6,2′-*O*-dibutyryl cyclic AMP	200	470
8-Bromo cyclic AMP	500	150
8-Benzylamino cyclic GMP	690	> 1000
8-Bromo cyclic IMP	760	> 1000
8-Bromo cyclic GMP	> 1000	> 1000
8-Benzylamino cyclic IMP	> 1000	> 1000
N^6-monobutyryl cyclic AMP	> 1000	160
2′-*O*-monobutyryl cyclic AMP	> 1000	10
Cyclic UMP	> 1000	> 1000
Cyclic CMP	> 1000	> 1000
2′-Deoxy cyclic AMP	> 1000	45
2′:3′-Cyclic GMP	> 1000	> 1000
2′:3′-Cyclic AMP	> 1000	> 1000
Cyclic GMP		> 1000
Cyclic AMP	> 1000	

* Assay conditions were as described in Fig. 1 except for the variation in the kind of compound used. I_{50} is defined as the concentrations of the compounds that inhibit 50 per cent of the phosphodiesterase activity. Cyclic GMP–PDE (0.04 μg) and cyclic AMP–PDE (4.0 μg) were from the gel electrophoresis [35] and DEAE-cellulose [34, 35] steps respectively. The concentration of either cyclic nucleotides was 1 μM.

AMP was the most potent inhibitor of the cyclic GMP–enzyme. 2′-*O*-monobutyryl cyclic AMP and 2′-deoxy cyclic AMP were the most potent inhibitors of cyclic AMP–PDE, with respective I_{50} values of 10 and 45 μM. No significant inhibiton of either enzyme by 2′:3′-cyclic GMP, 2′:3′-cyclic AMP, cyclic UMP and cyclic CMP was noted.

The differential sensitivity of the enzymes to inhibition by these agents was intriguing. Cyclic IMP and 2′-deoxy cyclic GMP were at least 1000 and 400 times more potent, respectively, in inhibiting cyclic GMP–PDE than in inhibiting cyclic AMP–PDE. Conversely, 2′-*O*-monobutyryl cyclic AMP and 2′-deoxy cyclic AMP were highly specific for inhibiting cyclic AMP–PDE. Deoxy cyclic GMP and deoxy cyclic AMP were found to competitively inhibit cyclic GMP–PDE and cyclic AMP–PDE respectively (Figs. 2 and 3). The apparent K_i values for deoxy cyclic GMP (1.7 μM) and deoxy cyclic AMP (24 μM) were 60 and 50 per cent lower, respectively, than the I_{50} values shown in Table 1.

The sensitivity of the cyclic GMP- and cyclic AMP-

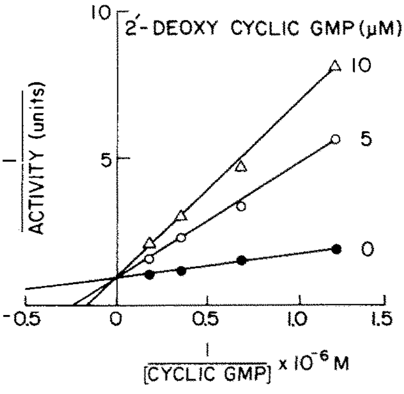

Fig. 2. Double reciprocal plot of the inhibition of cyclic AMP–PDE by 2′-deoxy cyclic GMP. The enzyme (0.04 μg) was from the gel electrophoresis step [35]. The concentrations of cyclic GMP used ranged from 0.83 to 5.8 μM. The data shown are the means of duplicate assays, and kinetic plots were determined by linear least square analysis.

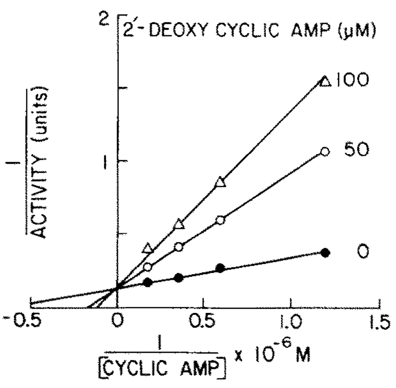

Fig. 3. Double reciprocal plot of the inhibition of cyclic AMP–PDE by 2′-deoxy cyclic AMP. The enzyme (4.0 μg) was from the DEAE-cellulose step [34, 35]. The concentrations of cyclic AMP used ranged from 0.83 to 5.8 μM. The data shown are the means of duplicate assays, and kinetic plots were determined by linear least square analysis.

Table 2. Differential inhibition by 2′-deoxy cyclic GMP and 2′-deoxy cyclic AMP of phosphodiesterases purified from the lung and in extracts of several tissues from guinea pigs*

Preparation and source of phosphodiesterase	Specific activity (units/mg protein) of enzyme hydrolyzing:			Per cent inhibition of cyclic GMP hydrolysis			Per cent inhibition of cyclic AMP hydrolysis		
	Cyclic GMP	Cyclic AMP	Ratio	Deoxy-cyclic GMP	Deoxy-cyclic AMP	Ratio	Deoxy-cyclic GMP	Deoxy-cyclic AMP	Ratio
Purified from lung†									
Cyclic GMP–PDE	26,956	1.0	26,960	65	2	33	N.D.‡	N.D.	N.D.
Cyclic AMP–PDE	95	696	0.13	N.D.	N.D.	N.D.	5	65	0.08
Crude extracts									
Lung	114 ± 2	109 ± 7	1.05	66 ± 3	3 ± 1	22.0	7 ± 2	63 ± 4	0.11
Ileum	139 ± 14	183 ± 15	0.76	50 ± 1	7 ± 2	7.1	11 ± 6	66 ± 1	0.17
Cerebellum	305 ± 28	211 ± 14	1.45	41 ± 5	6 ± 2	6.8	17 ± 7	58 ± 3	0.32
Mesenteric artery	73 ± 17	65 ± 9	1.12	50 ± 7	8 ± 4	6.3	26 ± 9	53 ± 7	0.49
Cerebrum	683 ± 47	355 ± 18	1.92	22 ± 1	7 ± 3	3.1	7 ± 4	39 ± 1	0.18
Aorta	45 ± 4	129 ± 20	0.35	64 ± 1	34 ± 9	1.9	47 ± 1	74 ± 5	0.64
Skeletal muscle	9 ± 1	140 ± 18	0.06	18 ± 5	10 ± 5	1.8	7 ± 5	72 ± 1	0.10
Heart	343 ± 33	474 ± 12	0.72	63 ± 3	58 ± 3	1.1	62 ± 5	71 ± 1	0.87

* Phosphodiesterase activity was assayed in the presence of 1 μM substrate concentrations as described in the text, and was carried out using enzyme protein (6–20 μg) and incubation times (6–15 min), which limited the hydrolysis of either cyclic nucleotide to 10–20 per cent. When included, the concentrations of deoxy cyclic GMP and deoxy cyclic AMP were 10 and 50 μM respectively. Values shown are the means (±S.E.) of at least three experiments with each assay performed in duplicate.

† The cyclic GMP–phosphodiesterase (0.04 μg) and cyclic AMP–phosphodiesterase (4.8 μg) were from the gel electrophoresis [35] and DEAE-cellulose [34, 35] steps respectively.

‡ Not determined.

hydrolyzing enzymes, present in crude extracts of several tissues from the guinea pig, to inhibition by deoxy cyclic GMP and deoxy cyclic AMP varied considerably within a tissue as well as between tissues (Table 2). The inhibition of the purified pulmonary enzymes was found to be qualitatively and quantitatively similar to that observed in crude extracts from the same tissue. The cyclic GMP-hydrolyzing activity present in extracts of the lung, aorta and cardiac ventricle was inhibited about 65 per cent by deoxy cyclic GMP. Deoxy cyclic AMP only slightly (less than 10 per cent) inhibited the cyclic GMP-hydrolyzing activity in extracts of most tissues, but higher degrees of inhibition (up to 58 per cent) by it were noted in extracts of the aorta and heart. The specificity index (per cent of inhibition of cyclic GMP hydrolysis by deoxy cyclic GMP/per cent of inhibition of cyclic GMP hydrolysis by deoxy cyclic AMP) for cyclic GMP hydrolysis was in a decreasing order: lung > ileum, cerebellum, mesenteric artery > cerebrum > aorta, skeletal muscle > heart. The degree of inhibition of cyclic AMP hydrolysis by deoxy cyclic AMP was 39–74 per cent for all tissue. The inhibition of cyclic AMP hydrolysis by deoxy cyclic GMP, however, was more variable, ranging from 7 to 62 per cent. Thus, the specificity index for cyclic AMP hydrolysis was in decreasing order: skeletal muscle, lung > ileum, cerebellum > cerebrum > mesenteric artery > aorta > cardiac ventricle.

Effect of aliphatic alcohols and various phosphodiesterase inhibitors on the activities of the purified pulmonary enzymes. Differential effects of alcohols on cyclic GMP–PDE and cyclic AMP–PDE are illustrated in Fig. 4. For example, ethanol (13 per cent v/v) and methanol (13 per cent v/v) were found to stimulate cyclic GMP–PDE 80 and 115 per cent, respectively, while conversely inhibiting cyclic AMP–PDE 75 and 50 per cent respectively. Systematic analysis of a series of aliphatic alcohols revealed that other alcohols were unable to stimulate cyclic GMP–PDE. Activation of the cyclic GMP–enzyme by either ethanol or methanol was due to increases in the V_{max} without altering the K_m for cyclic GMP (Fig. 5).

Various agents known to inhibit phosphodiesterase activities from other sources were studied for their effects on the purified pulmonary enzymes. The most

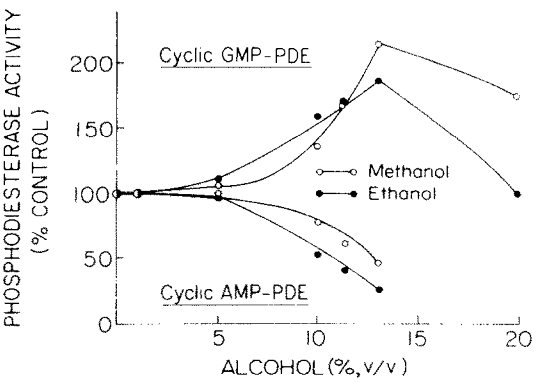

Fig. 4. Effect of methanol and ethanol on the activities of cyclic GMP–PDE and cyclic AMP–PDE. The cyclic GMP–PDE (0.03 μg) and cyclic AMP–PDE (4 μg) were from the gel electrophoresis [35] and DEAE-cellulose [34, 35] steps respectively. The assay was performed using a 1 μM concentration of cyclic GMP or cyclic AMP.

potent inhibitors of cyclic GMP–PDE were papaverine and 1-methyl-3-iso-butylxanthine (MIX) having I_{50} values of 3.5 and 4.2 μM respectively (Table 3). SQ 20,009 and papaverine were the most effective inhibitors of the cyclic AMP–PDE, with I_{50} values of 2 and 4 μM respectively. Generally, the agents were more effective inhibitors of the cyclic AMP–enzyme, with Ro 7-2956 and Ro 20-1724 being at least twenty times more potent in inhibiting the cyclic AMP–PDE than in inhibiting cyclic GMP–PDE. Only MIX demonstrated any significant specificity for cyclic GMP–PDE.

Kinetics of the inhibition of the cyclic GMP– and cyclic AMP–enzymes by various compounds were performed (figures not shown), and their kinetic data are summarized in Table 4. With the exception of mixed inhibition kinetics observed for SQ 20,009 on cyclic AMP–PDE, all other agents exhibited classic competitive inhibitory kinetics on both classes of phosphodiesterases. The apparent K_i values, while slightly lower, were in general agreement with the I_{50} values present in Table 3.

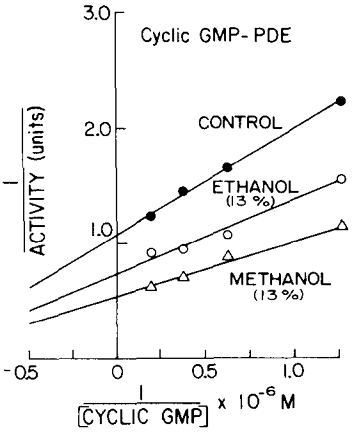

Fig. 5. Double reciprocol plot of the activation of cyclic GMP–PDE by methanol and ethanol. Cyclic GMP–PDE (0.03 μg) was from the gel electrophoresis step [35]. The concentrations of the cyclic GMP used ranged from 0.83 to 5.8 μM.

Table 3. Inhibition of cyclic GMP–PDE and cyclic AMP–PDE purified from guinea pig lung by various phosphodiesterse inhibitors*

	I_{50} (μM)		
Inhibitor	Cyclic GMP–PDE	Cyclic AMP–PDE	Ratio
Papaverine	3.5	4	0.9
1-Methyl-3-isobutylxanthine	4.2	24	0.2
SQ 20,009	15	2	7.5
Ro 20-1724	160	8	20.0
Sch 15280	800	300	2.7
Theophylline	1100	470	2.3
Caffeine	3200	1800	1.8
Ro 7-2956	> 1000	35	> 28.6

* Assay conditions were as described in the text. The reaction was carried out at 37° using either 1 μM cyclic GMP or cyclic AMP as substrate. The concentrations of the inhibitors used ranged from 10^{-6} to 10^{-2} M. The amounts and preparations of the enzymes were as indicated in Table 1. All drugs were dissolved in 10% dimethyl sulfoxide, and the final concentration of the solvent in the incubation was 1%, which was without effect on both classes of phosphodiesterases. All assays were performed in duplicate and the means of two separate determinations are presented.

Table 4. Summary of inhibition by various compounds of cyclic GMP–PDE and cyclic AMP–PDE purified from guinea pig lung*

	Cyclic GMP–PDE		Cyclic AMP–PDE	
Inhibitor	K_i (μM)	Type of inhibition	K_i (μM)	Type of inhibition
Cyclic IMP	1.1	Competitive	N.D.†	N.D.
2′-Deoxy cyclic GMP	1.7	Competitive	N.D.	N.D.
Papaverine	2.6	Competitive	2.6	Competitive
1-Methyl-3-isobutyl xanthine	4	Competitive	23	Competitive
SQ 20,009	9.8	Competitive	N.D.	Mixed
Theophylline	700	Competitive	540	Competitive
2′-Deoxy cyclic AMP	N.D.	N.D.	24	Competitive

* The enzymes were assayed in the presence of two different concentrations of the inhibitors that inhibit 20–60 per cent of the enzyme activity. Analysis of data was made using double reciprocal plots. The concentration of either cyclic GMP or cyclic AMP used ranged from 0.83 to 5.8 μM. The amounts and preparations of the enzymes are indicated in Table 1.

† Not determined.

DISCUSSION

It was of interest that the three butyryl derivatives of cyclic GMP and cyclic AMP exhibited the same relative order of potency as inhibitors of cyclic GMP–PDE and cyclic AMP–PDE respectifely. Similar order of potency has been reported for the inhibition of cyclic AMP-hydrolyzing enzymes from other tissues by the butyryl derivatives of cyclic AMP [28]. It would appear from the present data, and the results of others [28, 33], that the effects of dibutyryl derivatives of cyclic GMP and cyclic AMP on various tissue preparations may be due, in part, to their ability, or more importantly the ability of the monoacylated metabolites, to inhibit phosphodiesterases. It should be mentioned that a somewhat different order of potency was observed for the butyryl derivatives of cyclic GMP in activating cyclic GMP-dependent protein kinase from guinea pig lung [38].

That 8-substituted derivatives of the cyclic nucleotides were poor inhibitors of cyclic GMP–PDE is consistent with the observation by others using extracts of rabbit lungs [29] and rat brains [39]. Those derivatives were not hydrolyzed by cyclic GMP–PDE [35]. It is worth mentioning that 8-bromo cyclic GMP has recently been shown to be the most potent activator of cyclic GMP-dependent protein kinase from the guinea pig fetal lung [38] and fetal calf heart [40]. Deoxy cyclic GMP was shown to be a very potent inhibitor of the cyclic GMP–PDE (see Table 1) and yet is a very weak activator (K_a, 1×10^{-4} M) of pulmonary cyclic GMP-dependent protein kinase (J. F. Kuo, unpublished observations). These data suggest a distinct difference in the active sites of cyclic GMP–PDE and cyclic GMP-dependent protein kinase.

Of the xanthine derivatives studied, MIX was more potent in inhibiting either class of phosphodiesterase when compared to theophylline and caffeine. This is in agreement with the reports of others using different phosphodiesterase preparations [33, 41, 42]. That MIX was five times more potent in inhibiting cyclic GMP–PDE than in inhibiting cyclic AMP–PDE (see Table 4) is interesting, since it has recently been reported to be more potent in inhibiting a similar enzyme from pig coronary artery [33]. Other compounds tested were more selective in inhibiting cyclic AMP–PDE, the most selective of which were the 4-(benzyl)-2-imidazolidinones (Ro 7-2956 and Ro 20-1724), which have previously been shown to inhibit more specifically the cyclic AMP hydrolysis than the cyclic GMP hydrolysis, using the enzymes from the cerebral cortex [32]. For the most part, these compounds were found to inhibit both cyclic GMP–PDE and cyclic AMP–PDE in a competitive manner. It is intriguing that compounds of such diverse structure are able to alter the catalytic sites of two enzymes which so readily discriminate such similar structures as cyclic GMP and cyclic AMP. It might be speculated that the apparent competitive inhibition arises through the binding of these agents to some site, perhaps common to both enzymes, that is in close proximity to the active site. The relative ability to inhibit the respective enzymes would then be governed by the ability of the compounds to cause "induced unfit" of the active site to the respective substrates, i.e. cyclic GMP and cyclic AMP.

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