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Properties and Drug Responsiveness of Cyclic Nucleotide Phosphodiesterases of Rat Lung

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SUMMARY

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Cyclic 3′,5′-AMP phosphodiesterase and cyclic 3′,5′-GMP phosphodiesterase of rat lung were found predominantly in the 100,000 × *g* supernatant fraction. Both enzymes had biphasic Hofstee plots, with apparent K_m values of 0.3 μM and 115 μM for cyclic AMP, and 1.5 μM and 19 μM for cyclic GMP. Electrophoretic analysis of the soluble supernatant fraction of lung on a preparative polyacrylamide gel column showed two stable peaks of phosphodiesterase activity. The first peak, which had relatively little phosphodiesterase activity, hydrolyzed only cyclic AMP, whereas the other peak hydrolyzed both cyclic AMP and cyclic GMP. Both peaks of cyclic AMP phosphodiesterase activity of lung had biphasic Hofstee plots. The first had apparent K_m values of 2.9 μM and 200 μM, and the second had K_m values of 1.9 μM and 190 μM; the cyclic GMP peak had a single K_m of 7 μM. These peaks of phosphodiesterase were markedly different in their response to drugs; the first peak was highly resistant to pharmacological inhibition while the second was sensitive to several phosphodiesterase inhibitors. For example, the inhibitor constant (K_i) of trifluoperazine was approximately 1000 μM for the first peak but only about 50 μM for the second peak. In this second peak, both cyclic AMP and cyclic GMP phosphodiesterase activities were inhibited to a similar extent by a variety of inhibitors. Neither the cyclic AMP nor cyclic GMP phosphodiesterase of crude or electrophoretically purified fractions was significantly activated by a heat-stable factor derived from rat cerebrum or rat lung. However, the lung does contain an activator of phosphodiesterase which is capable of stimulating brain phosphodiesterase. The differential effects of inhibitors on the multiple forms of phosphodiesterase suggest that the concentration of cyclic nucleotides in lung may be selectively altered by appropriate pharmacological agents.

INTRODUCTION

Increased knowledge of the role of cyclic nucleotides in the function of cells under normal and pathological conditions has led to the possibility that pharmacological control of cyclic nucleotide concentrations may have therapeutic value (1). One way in which intracellular cyclic nucleotide concentrations can be manipulated is by altering the activity of cyclic nucleotide phosphodiesterases in a given tissue or cell type. Evidence that cyclic nucleotide phosphodiesterases exist in multiple forms or isoenzymes (2–9), and that these enzymes

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are differentially affected by pharmacological agents (10), provides a basis for the control of cyclic nucleotide concentrations in specific cell types.

Recent studies indicate that cyclic nucleotides are involved in certain functions of lung, in particular, the immune response (11) and the anaphylactic reaction (12). This suggests that control of cyclic nucleotide concentrations in the lung may permit an alteration in the course of the anaphylactic reaction. Accordingly, we have studied the properties of the cyclic 3′,5′-AMP and cyclic 3′,5′-GMP phosphodiesterases of rat lung and have evaluated their interaction with a variety of pharmacological inhibitors and an endogenous activator of phosphodiesterase.

METHODS

Tissue preparation. All experiments were performed with lung excised from 200–250-g male rats (Sprague-Dawley) killed by cervical dislocation. The heart and lungs were rapidly removed, and the lungs were freed of blood by perfusion with 0.9% NaCl through the aorta. Bronchial tissue was removed and discarded, as were areas of the lung which were not well perfused. The remaining lung tissue was homogenized for 5 sec in 3 volumes of 0.32 M sucrose in a Brinkmann Polytron. The homogenate was centrifuged at 100,000 × *g* for 60 min, the supernatant and particulate fractions were separated, and the particulate fraction was resuspended in glycylglycine buffer, 150 μM (pH 8.0), equal in volume to the original homogenate.

Separation of subcellular fractions. Tissue was homogenized in 0.32 M sucrose, and the subcellular fractions were prepared as described by Gray and Whittaker (13) and DeRobertis *et al.* (14).

Separation of multiple forms of phosphodiesterase. The 100,000 × *g* supernatant fraction was placed on a preparative polyacrylamide gel electrophoresis column prepared as previously described (6), and subjected to 60 mamp of current for 18 hr. The fractions eluted from the column were collected and analyzed for both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities.

Cyclic nucleotide phosphodiesterase assays. Cyclic AMP phosphodiesterase activity was measured by the method of Weiss, Lehne, and Strada (15), and cyclic GMP phosphodiesterase was measured by the method of Fertel and Weiss (16).

Preparation of activator. Phosphodiesterase activator was obtained from rat lung and cerebrum by the procedure of Cheung (17). The tissue was homogenized in 3 volumes of distilled water, brought to pH 5.9 with 1 N HCl, and centrifuged at 13,000 × *g* for 30 min, and the precipitate was discarded. The supernatant fluid was heated in a boiling water bath, centrifuged, heated and centrifuged again, and finally dialyzed for 24 hr against 20 mM glycylglycine buffer, pH 7.5. The activator was stored at −20° until used.

Determination of K_m values. Enzyme activity at different substrate concentrations was plotted according to Hofstee (18). The line of best fit and the slope of this line (which is the K_m value) were computed by linear regression analysis. However, the best fit for the biphasic curves required a subjective evaluation of the point of deflection. In addition, it is important to point out that the kinetic constants obtained at high substrate concentrations result from the combined activities of the high- and low-K_m enzymes [for a discussion of this point, see Weiss and Strada (19)]. Therefore, the K_m values which we report should be regarded only as estimates.

Determination of K_i values. Enzyme activity was determined at several concentrations of substrate and inhibitor. The data were plotted according to Dixon (20). The line of best fit was calculated by linear regression analysis, and the K_i value was estimated from the median point of intersection of the lines. Determinations of activity were based on standard curves run in the presence of the inhibitor being tested in order to control for the possible effects of these agents on the assay system. Since the Dixon plot does not permit the determination of the type of inhibition under certain conditions (21), the data were also examined using reciprocal plots, as described by Lineweaver and Burk (22).

Protein determination. Protein was de-

termined according to Lowry *et al.* (23), using bovine serum albumin as a standard.

Materials. Firefly luciferin-luciferase was obtained from du Pont. The enzymes for phosphodiesterase analysis were obtained from Boehringer/Mannheim, and all other chemicals and reagents were obtained from Sigma Chemical Company. Trifluoperazine was kindly supplied by Smith Kline & French, and SQ 20,009 [1-ethyl-4-(isopropylidenehydrazino)-1*H*-pyrazolo-(3,4-*b*)pyridine-5-carboxylic acid ethyl ester hydrochloride], by Squibb.

RESULTS

Subcellular distribution of cyclic AMP and cyclic GMP phosphodiesterase activities of rat lung. The specific activity and percentage of total recovered activity of phosphodiesterase in each subcellular fraction of lung are shown in Table 1. As can be seen, the soluble fraction of lung contained the highest total activity and the highest specific activities of both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase.

Separation of multiple forms of phosphodiesterase by polyacrylamide gel electrophoresis. Figure 1 shows the electrophoretic pattern of cyclic AMP and cyclic GMP phosphodiesterase activity in a 100,000 × *g* supernatant fraction of rat lung. Under the conditions of this separation procedure, there was an initial, relatively small peak of cyclic AMP phosphodiesterase activity, which corresponded to peak I obtained from similarly treated preparations of rat cerebellum and cerebrum (6, 9, 10). The first peak was followed by a much larger peak of cyclic AMP phosphodiesterase activity; the characteristics of this peak closely resembled those of peak III of rat cerebrum and cerebellum with respect to electrophoretic mobility (9). The two eluted peaks therefore were

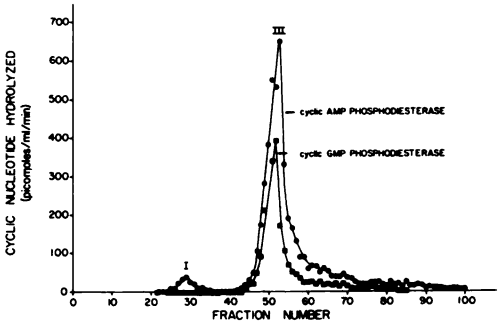

FIG. 1. *Cyclic AMP and cyclic GMP phosphodiesterase activities of rat lung supernatant fraction separated by polyacrylamide gel electrophoresis*

A rat lung was prepared as described in the text, and 2 ml of the 100,000 × *g* supernatant fraction were placed on a preparative gel electrophoresis column. The electrophoresis was conducted as described in METHODS. Fractions (2 ml each) were collected and analyzed for total (high- plus low-K_m) phosphodiesterase activity, using 400 μM cyclic AMP or 200 μM cyclic GMP. Each point represents the mean of two enzyme determinations on a single sample.

TABLE 1

Cyclic AMP and cyclic GMP phosphodiesterases in subcellular fractions of rat lung

Subcellular fractions were obtained and analyzed for cyclic AMP phosphodiesterase activity at a substrate concentration of 400 μM and for cyclic GMP phosphodiesterase activity at a substrate concentration of 200 μM. Phosphodiesterase activity and protein were determined as described in METHODS. Each number represents the mean specific activity of three lung preparations ± standard error. The percentage of total recovered activity in a given fraction was calculated by dividing its total activity by the total activity in all four subcellular fractions. The total recovered activity relative to the total activity in the starting homogenate was approximately 80% for both the cyclic AMP and cyclic GMP phosphodiesterases.

Fraction	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	nmoles cyclic AMP hydrolyzed/ mg protein/min	*% total recovered activity*	*nmoles cyclic GMP hydrolyzed/ mg protein/min*	*% total recovered activity*
Homogenate	5.3 ± 0.9		9.1 ± 4.3	
900 × *g* sediment	1.5 ± 0.4	17.7	1.1 ± 0.4	21
11,500 × *g* sediment	3.0 ± 1.0	8.2	1.2 ± 0.02	5.5
100,000 × *g* sediment	4.4 ± 2.1	12.2	4.9 ± 2.4	20.2
100,000 × *g* supernatant	17.6 ± 3.0	61.9	10.0 ± 1.9	53.3

designated I and III, respectively. Cyclic GMP phosphodiesterase activity was found only in those fractions which also displayed peak III cyclic AMP phosphodiesterase activity. The high degree of correspondence between cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in these fractions may indicate that the two activities are found in the same molecular form.

In some experiments another small peak of activity appeared in fractions 65–70. This peak, which exhibited both cyclic AMP and cyclic GMP phosphodiesterase activity, was extremely unstable and therefore difficult to study. Earlier studies showed that a similar highly purified fraction of brain phosphodiesterase (peak IV) was also very unstable (9).

Kinetic properties of cyclic AMP and cyclic GMP phosphodiesterases of crude and purified preparations of rat lung. A kinetic analysis of cyclic AMP and cyclic GMP phosphodiesterases was carried out on the crude homogenate, 100,000 × *g* sediment, and 100,000 × *g* supernatant fractions, as well as on the purified enzyme peaks eluted from the preparative column (Table 2). Both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in all three crude fractions displayed the biphasic kinetic pattern described by Thompson and Appleman (2), suggesting that the phosphodiesterases may exhibit negative cooperativity. The two purified peaks of cyclic AMP phosphodiesterase activity had biphasic kinetic patterns, but the cyclic GMP phosphodiesterase in peak III had only a single K_m value. The apparent K_m values for each of these peaks and the crude fractions are shown in Table 2. With the exception of the low-K_m (K_{m1}) activity of the soluble cyclic AMP phosphodiesterase, the apparent K_m values for cyclic GMP phosphodiesterase are lower, in each case, than the corresponding values for cyclic AMP phosphodiesterase. The K_m values for the cyclic AMP phosphodiesterase of the separated peaks are higher than the K_m values obtained for the cyclic AMP phosphodiesterase of the unfractionated soluble preparation.

TABLE 2

K_m values of cyclic nucleotide phosphodiesterases of rat lung

Fractions were obtained as described in METHODS. Each study was carried out using at least 14 concentrations of substrate (1.0–500 μM). The low-K_m enzyme is designated K_{m1}, and the high-K_m enzyme, K_{m2}.

Fraction	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	K_{m1}	K_{m2}	K_{m1}	K_{m2}
	μM	μM	μM	μM
Homogenate	4.7	150	1.6	44
Soluble	0.30	110	1.4	19
Particulate	8.0	130	3.6	54
Separated peaks from soluble fraction				
Peak I	2.9	200		
Peak III	1.9	180	6.7	

Effect of pharmacological agents on phosphodiesterases of rat lung. A variety of pharmacological agents were studied for their effect on the soluble cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase of rat lung. A typical dose-response curve for one of these inhibitors is shown in Fig. 2. Although concentrations of trifluoperazine up to 80 μM inhibited both phosphodiesterases to a similar extent, high concentrations of the inhibitor had less effect on the cyclic AMP phosphodiesterase activity than on the cyclic GMP phosphodiesterase activity. This suggests that a small fraction of the cyclic AMP phosphodiesterase present in the sample is resistant to inhibition.

Figure 3, which shows the effect of trifluoperazine on cyclic AMP phosphodiesterase of a purified peak III fraction of rat lung, illustrates the procedure used to calculate the K_i values for phosphodiesterase inhibitors and emphasizes the complex nature of the enzymes. Two sets of intersecting lines may be drawn through the experimental points for each concentration of substrate studied. Since the exact point at which the first series of lines ended and the second began was not always as obvious as in the illustrated example, we

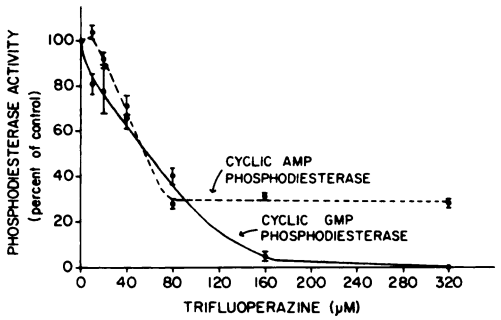

FIG. 2. *Effect of trifluoperazine on cyclic AMP and cyclic GMP phosphodiesterase activities of rat lung supernatant fraction*

Rat lung supernatant fraction was prepared as described in METHODS. Each sample was incubated with 400 μM cyclic AMP or 200 μM cyclic GMP and the indicated concentration of trifluoperazine. Five samples were tested at each inhibitor concentration. The mean activity ± standard error was calculated for each condition and expressed as a percentage of the control. The cyclic AMP phosphodiesterase activity in the absence of inhibitor was 8.2 nmoles of cyclic AMP hydrolyzed per milligram of protein per minute, and that for the cyclic GMP phosphodiesterase was 2.8 nmoles of cyclic GMP hydrolyzed per milligram of protein per minute.

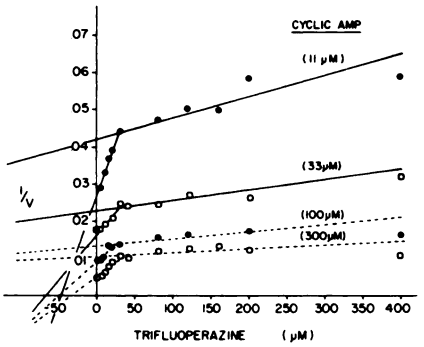

FIG. 3. *Effect of trifluoperazine on cyclic AMP phosphodiesterase activity of rat lung (peak III)*

Cyclic AMP phosphodiesterase (peak III) was obtained as described in METHODS. Phosphodiesterase activity was determined in the presence of 12 concentrations of trifluoperazine (from 5 to 400 μM) and four concentrations of cyclic AMP (11–300 μM). Standard curves were generated in the presence of the inhibitor to ensure that it did not interfere with the assay. Each point is the mean of duplicate determinations. The line of best fit was calculated by a linear regression analysis, and the median point of intersection was used to determine the K_i value.

have chosen to calculate and report in the subsequent tables only the low K_i values.

Table 3 shows the calculated K_i values of several pharmacological agents on the cyclic AMP and cyclic GMP phosphodiesterases of a soluble supernatant fraction of rat lung. As can be seen, the agents examined have widely varying potencies. The most potent inhibitors studied, papaverine and SQ 20,009, had K_i values of about 10–30 μM. In contrast, theophylline, the least effective agent, had a K_i value which was approximately 10 times higher. The order of potency was the same for both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase, indicating that these two enzyme activities respond in a similar fashion to inhibitors.

A similar study of the isozymes of phosphodiesterase which were separated from the supernatant fraction of rat lung indicated that the purified phosphodiesterase peaks had markedly different sensitivities to pharmacological agents. For example, papaverine inhibited the second peak (peak III) to a much greater extent than the first peak (peak I) (Fig. 4). In fact, the first peak was extremely resistant to the inhibitory effects of all pharmacological agents examined. The inhibition of the cyclic GMP phosphodiesterase activity found in peak III (Fig. 5) was similar to the inhibition of cyclic AMP phosphodiesterase found in the same peak.

TABLE 3

Effect of inhibitors on cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase of 100,000 × g supernatant fraction of rat lung

Cyclic nucleotide phosphodiesterase activity was tested in quadruplicate at five different inhibitor concentrations for each of four different substrate concentrations. The data were plotted according to Dixon (20). The median point of intersection determined the K_i value. Each number represents the K_i value calculated from a separate enzyme preparation. Theophylline, papaverine, and SQ 20,009 were competitive inhibitors at low concentrations, and mixed or noncompetitive at high concentrations. Trifluoperazine produced a mixed type of inhibition.

Inhibitor	K_i	
	Cyclic AMP phosphodiesterase	Cyclic GMP phosphodiesterase
	μM	μM
Theophylline	350	150; 250; 200
Trifluoperazine	60; 39	22; 20; 47
Papaverine	22; 7; 8	8
SQ 20,009	15	15; 27

The K_i values for a series of compounds toward the separated peaks of phosphodiesterase are given in Table 4. The cyclic AMP phosphodiesterase found in peak I was extremely resistant to inhibition by all the agents studied, papaverine being the most potent inhibitor and theophylline the least potent. Both the cyclic AMP and cyclic GMP phosphodiesterase activities of peak III were inhibited more readily than the activity of peak I. In some cases the inhibitory effect of a given agent on peak III was an order of magnitude greater than its effect on peak I. As with the enzymes in the unpurified supernatant fraction, SQ 20,009 and papaverine were the most effective inhibitors. Cyclic GMP, which in most systems is a competitive inhibitor of cyclic AMP phosphodiesterase activity (10, 24, 25), was more potent inhibitor than theophylline and less effective than either papaverine or SQ 20,009 in this system. Cyclic AMP was a comparatively weak inhibitor of cyclic GMP phosphodiesterase.

Effect of a heat-stable phosphodiesterase activator on rat lung phosphodiesterase activity. An activator of phosphodiesterase was isolated from both rat lung and rat brain and tested for its effect on the cyclic AMP phosphodiesterase prepared from the 100,000 × g soluble fractions of rat lung and rat cerebrum (Fig. 6). It is apparent that rat lung contains an activator which is capable of stimulating rat cerebrum cyclic AMP phosphodiesterase by 3–4-fold. This effect was also seen when activator

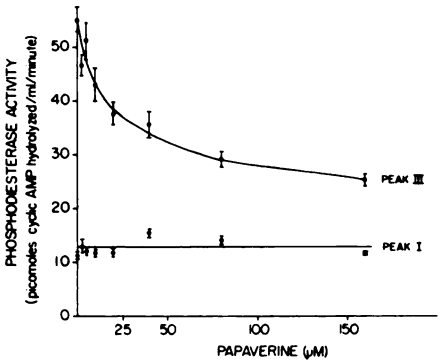

FIG. 4. *Effect of papaverine on cyclic AMP phosphodiesterase activity of electrophoretically separated peaks I and III of rat lung*

Fractions containing cyclic AMP phosphodiesterase activity were eluted from a preparative polyacrylamide gel electrophoresis column as described in METHODS. Five samples of each peak were assayed at each inhibitor concentration at a substrate concentration of 400 μM. The results are expressed as means ± standard errors.

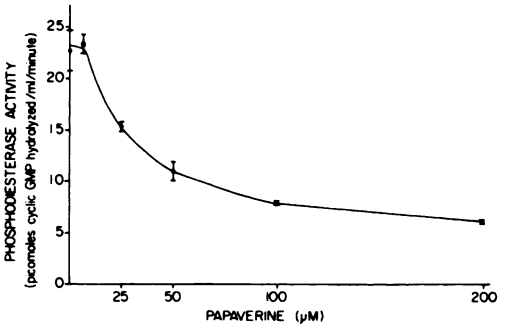

FIG. 5. *Effect of papaverine on cyclic GMP phosphodiesterase activity of electrophoretically separated peak III of rat lung*

A fraction containing cyclic GMP phosphodiesterase activity was eluted from a preparative column and analyzed as in Fig. 4, except that the substrate concentration was 200 μM.

TABLE 4

K_i values of inhibitors for cyclic nucleotide phosphodiesterase isozymes of rat lung

Conditions were the same as described in Table 3 and METHODS. Each number represents the K_i value calculated from a separate enzyme preparation. All inhibitors were competitive at low concentrations and mixed or noncompetitive at high concentrations.

Inhibitor	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase, peak III
	Peak I	Peak III	
	μM	μM	μM
Theophylline	940; 1430	500; 310	270; 150
Trifluoperazine	680; 1100	36; 68	53; 10
Papaverine	170	29	42
SQ 20,009	550	16	27
Cyclic GMP	780; 1170	65; 100	
Cyclic AMP			550

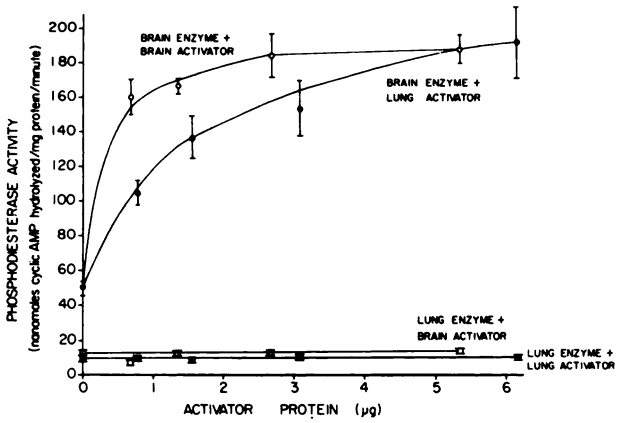

FIG. 6. *Effect of activator derived from rat lung and cerebrum on cyclic AMP phosphodiesterase activity of supernatant fraction from rat lung and cerebrum*

The activator and cyclic AMP phosphodiesterase were obtained as described in METHODS. The effect of varying amounts of both activators on the phosphodiesterase from both tissue sources was tested under conditions (400 μM substrate; 40 μM Ca^{++}; 3 mM Mg^{++}) which provide maximum stimulation.[2] Each point represents the mean of five individual determinations ± standard error.

from rat cerebrum was added to the enzyme from rat cerebrum. However, neither activator preparation effectively stimulated the cyclic AMP phosphodiesterase (Fig. 6) or cyclic GMP phosphodiesterase (results not shown) prepared from the supernatant fraction of rat lung. This lack of activatability of the phosphodiesterases of lung was confirmed in experiments in which activator was added to each fraction eluted from the polyacrylamide gel column and cyclic AMP and cyclic GMP phosphodiesterase activities were determined. No significant stimulation of the phosphodiesterase of any of the fractions was noted.

The relative lack of effect of activator from rat lung on the soluble cyclic AMP phosphodiesterase from rat lung may be explained as follows. First, it is possible that the activatable enzyme from lung is in the 100,000 × *g* particulate fraction of the cell, rather than in the supernatant fraction; second, it is possible that the soluble enzyme from rat lung may already be maximally stimulated and therefore may be unresponsive to the additional activator. To investigate the first possibility, the 100,000 × *g* particulate fraction of rat lung was incubated with several concentrations of activator isolated from rat lung. The particulate fraction was not stimulated by the activator (results not shown).

To determine whether the soluble enzyme was already maximally activated, we took advantage of the requirement for Ca^{++} in activating phosphodiesterase (26–31). The soluble cyclic AMP phosphodiesterase from rat cerebrum and rat lung was isolated and incubated under four conditions: with no addition (untreated); with 300 μM EGTA;[3] with enough activator to give maximal stimulation; and with the activator plus EGTA. Experiments were performed with activator prepared from brain and lung; the results obtained with both these activator preparations were similar. The data indicate that the addition of activator to the preparation from cerebrum gave a 3-fold increase in phosphodiesterase activity (Fig. 7A). When EGTA was added to the same preparation, the activity was reduced by 40%. This indicated that the preparation was already partially activated. Finally, the addition of EGTA to the activated enzyme completely prevented the activation, indicating that the concentration of EGTA was sufficient to prevent activation by either endogenous or exogenous activator.

A similar protocol was used to deter-

[2] R. Levin and B. Weiss, unpublished observations.

[3] The abbreviation used is: EGTA, ethylene glycol bis(β-amino ethyl ether)-*N*,*N*′-tetraacetic acid.

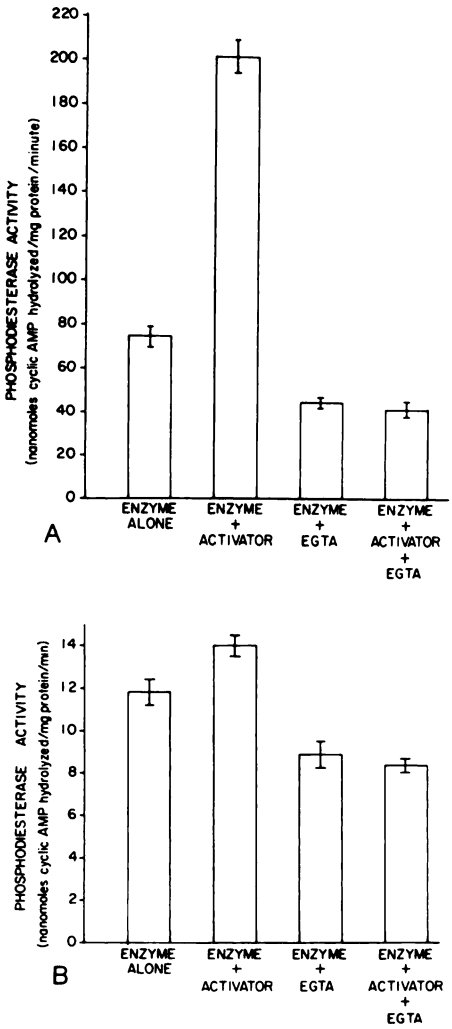

FIG. 7. *Effect of EGTA and activator on cyclic AMP phosphodiesterase of rat cerebrum (A) and rat lung (B)*

The 100,000 × *g* supernatant fraction of rat cerebrum or lung was obtained as described in METHODS. Samples were analyzed for cyclic AMP phosphodiesterase activity in the presence of 40 μM Ca^{++} and 400 μM substrate. The conditions of incubation were: enzyme alone; enzyme plus an amount of activator prepared from brain which had previously been shown to give maximal activation (7.7 μg of protein); enzyme plus 300 μM EGTA; and enzyme plus activator and EGTA. Each column represents the mean of five samples ± standard error.

mine the degree of activation of the rat lung cyclic AMP phosphodiesterase in the supernatant fraction (Fig. 7B). When activator was added to this preparation, there was less than a 20% increase in activity, which confirms the relative lack of activatability of the enzyme in the lung supernatant fraction. EGTA reduced activity slightly (about 30%), indicating that the enzyme might have already been activated to a small extent. The maximum activation obtainable in this preparation, as determined by dividing the activity of the activated enzyme by the activity of the EGTA-treated, or unactivated enzyme, was less than 60%. In comparison, under similar conditions brain enzyme was activated by more than 400%. On the basis of these experiments, it may be inferred that the phosphodiesterase from rat lung, unlike the enzyme from rat brain, can be activated only to a negligible extent.

DISCUSSION

Prior work with the phosphodiesterases of lung has been limited largely to that of Hitchcock (32, 33), who examined the kinetic parameters and ion and drug responsiveness of the cyclic AMP phosphodiesterase of guinea pig lung. There is no published information which deals either with the cyclic GMP phosphodiesterase or with chromatographically separated phosphodiesterase isozymes from lung.

The present studies show that the cyclic AMP and cyclic GMP phosphodiesterase activities of rat lung have similar subcellular distribution patterns (Fig. 1). That the majority of the cyclic AMP phosphodiesterase is found in the soluble cell fraction has been established for guinea pig lung (33) as well as for other tissues (24, 34–37).

The similar subcellular distribution patterns of cyclic GMP phosphodiesterase and cyclic AMP phosphodiesterase, and their coincidence after electrophoresis, suggest that the two activities may be found in the same enzyme molecule.

Kinetic studies of the unpurified cyclic GMP and cyclic AMP phosphodiesterases indicate that both enzymes have two apparent K_m values. Similar results have been obtained in many other tissues (2, 5, 37–41). The low K_m value for cyclic AMP in rat lung is 2 orders of magnitude less than that found by Hitchcock in the guinea pig lung (32). The difference may be due to either species or methodological variations.

A kinetic analysis of the phosphodiesterases following electrophoretic separation indicated that the purified peaks of cyclic AMP phosphodiesterase maintain their biphasic pattern. This contrasts with the results from previous studies, in which monophasic kinetics was observed following electrophoretic separation of cerebrum and cloned glial cells (9). The monophasic kinetics shown by the purified cyclic GMP phosphodiesterase contrasts with the biphasic kinetics seen with the unpurified enzyme. The biphasic kinetic pattern displayed by the purified cyclic AMP phosphodiesterase isozyme may be due to the presence of a second enzyme which is not distinguishable by the purification procedure used. A more likely explanation is the one thoroughly discussed by Russell *et al.* (42), who demonstrated that cyclic AMP phosphodiesterase may be a negatively cooperative enzyme.

The multiple forms of phosphodiesterase are differentially affected by pharmacological agents (9, 10). Our results with the phosphodiesterases of lung confirm these observations. Peak I cyclic AMP phosphodiesterase activity is highly resistant to all the inhibitors examined. In contrast, peak III cyclic AMP phosphodiesterase is readily inhibited by papaverine and SQ 20,009. Peak III cyclic GMP phosphodiesterase, which has the same electrophoretic mobility as peak III cyclic AMP phosphodiesterase, responds to the inhibitors in an identical fashion, which suggests that both cyclic AMP and cyclic GMP phosphodiesterase activities are found in the same molecule. A graphic demonstration of the effect of one of the inhibitors, papaverine (Figs. 4 and 5), gives further evidence that the cyclic GMP isozyme and one of the cyclic AMP isozymes (peak III) react in a similar way to inhibitors, while the other cyclic AMP phosphodiesterase isozyme (peak I) is much less sensitive to inhibition.

The pattern of phosphodiesterase in lung, and the response of these isozymes to inhibitors, explain the effect of trifluoperazine on the enzymes in the supernatant fraction (Fig. 2). Peak I, which contains only cyclic AMP phosphodiesterase activity, provides a small fraction of the cyclic AMP-hydrolyzing capacity of the lung. It is also insensitive to inhibition. However, peak III, which constitutes most of the enzyme activity, is sensitive to inhibition. Thus, at low drug concentrations, the activity of the more sensitive enzyme (peak III), which contains both cyclic AMP and cyclic GMP phosphodiesterase activities, is markedly reduced. Higher concentrations of trifluoperazine completely inhibit peak III. Therefore all of the cyclic GMP phosphodiesterase and most of the cyclic AMP phosphodiesterase activity is lost. However, at these high concentrations, the less sensitive cyclic AMP-hydrolyzing enzyme (peak I) is unaffected, and a small percentage of the activity of cyclic AMP phosphodiesterase remains.

The presence of a phosphodiesterase activator in lung is not surprising, since it was shown previously that the activator is found in many tissues (43). However, this activator from lung is considerably less potent than that from cerebrum (Fig. 6.) This may be due to functional differences in the activator from the two tissues, or to differences in the specific activities of lung and cerebral activator. Our present data do not permit us to distinguish between these possibilities.

Although the lung clearly contains a phosphodiesterase activator, it contains little or no phosphodiesterase capable of being activated. For example, an amount of activator which produced a 350% increase in the activity of brain cyclic AMP phosphodiesterase caused less than a 50% increase in the activity of the phosphodiesterase from lung. These results are consistent with the observation that, based on its electrophoretic pattern, lung does not contain a detectable peak II phosphodiesterase, the only major form which can be activated (6, 9, 10). The slight activation which was observed may be a reflection of the fact that lung contains more than 20 types of cells (44, 45), only a small proportion of which may have an activatable enzyme.

There are at least two explanations for the finding that lung contains a significant amount of activator but has little activatable enzyme. First, it is possible that the lung enzyme is activated by a factor which

is not calcium-dependent. This is unlikely, since activator from all tissues studied requires Ca^{++} (26–31). It is also possible that the material which we isolated from the lung and which has the ability to activate phosphodiesterase has another, as yet unknown, function in the cell.

In conclusion, the different patterns of the phosphodiesterases of lung compared with other tissues, and the different responses of the two lung cyclic AMP phosphodiesterase isozymes to a variety of pharmacological agents, are further evidence for the possibility of pharmacological control of individual phosphodiesterase isozymes. This, in turn, would permit selective alteration of cyclic nucleotides in individual tissues and cell types.

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