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Selective Alteration of the Activity of the Multiple Forms of Adenosine 3′,5′-Monophosphate Phosphodiesterase of Rat Cerebrum

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SUMMARY

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Four different peaks of cyclic 3′,5′-AMP phosphodiesterase activity were isolated from the supernatant fraction of rat cerebral homogenates using preparative acrylamide gel electrophoresis. These peaks of activity, designated I-IV according to the order in which they emerged from the column, were exposed to various inhibitors of phosphodiesterase and to an activator of phosphodiesterase isolated from brain. The activator stimulated peak II phosphodiesterase about 10-fold but had no effect on the other peaks. The inhibitors also had differential effects on the phosphodiesterases, the most notable being trifluoperazine, which preferentially inhibited the activated peak II. Trifluoperazine sulfoxide and promethazine had less than 10% of the inhibitory activity of trifluoperazine on peak II. The inhibitors differed not only in the specific phosphodiesterase which they inhibited but also in their mechanism of action. Thus theophylline, cyclic GMP, and low concentrations of papaverine acted as competitive inhibitors, whereas the inhibition produced by trifluoperazine was neither purely competitive nor purely noncompetitive. High concentrations of papaverine produced noncompetitive inhibition. Theophylline and papaverine inhibited the activated and unactivated peak II equally, but trifluoperazine inhibited the activated peak II by more than 90% in concentrations (40 μM) that had little effect on the unactivated enzyme. This inhibition of peak II phosphodiesterase by trifluoperazine could be prevented by increasing the concentration of activator. Our results suggest that the selectivity of phosphodiesterase inhibitors may be due to the different patterns and ratios of multiple forms of phosphodiesterase in different tissues.

INTRODUCTION

The intracellular concentration of adenosine 3′,5′-monophosphate and other cyclic nucleotides is controlled, in part, by a group of cyclic nucleotide phosphodiesterases. These enzymes exist in several molecular forms, each with its own particular properties, substrate specificities, and cellular distribution (1–12).

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The activity of the phosphodiesterases can be altered by a wide variety of chemical agents. Several compounds with diverse chemical structures have been shown to inhibit phosphodiesterase activity (13–22), and an endogenous heat-stable protein has been shown to activate phosphodiesterase (8, 23, 24). Recent reports show that drugs can differentially inhibit the activity of phosphodiesterase obtained from different tissues (17, 20, 25–29). These effects might be the result of a differential inhibitory action of drugs on the multiple forms of phosphodiesterase found in these tissues. This possibility is supported by the present results, which show that an endogenous activator and several known inhibitors of phosphodiesterase have differential effects on the multiple forms of cyclic AMP phosphodiesterase isolated from rat cerebrum.

METHODS

Male Sprague-Dawley rats weighing approximately 200 g were used in these experiments. The animals were decapitated, and the cerebral hemispheres were scraped free of white matter. The remaining tissue, consisting mainly of cortical gray matter, was homogenized in 0.32 M sucrose, sonicated in a Branson S125 Sonifier at peak intensity for 15 sec, and centrifuged at 100,000 × g for 1 hr. A portion of the soluble supernatant fluid was then placed on a preparative polyacrylamide gel column, and electrophoresis was conducted for 18 hr as described previously (8). The material eluted from the gel column was collected in 1-ml fractions, and each fraction was analyzed for phosphodiesterase activity. The effects of an activator of phosphodiesterase and of various inhibitors of phosphodiesterase on the isolated peaks of phosphodiesterase were then examined. The peaks of activity thus isolated apparently represent different molecular forms of phosphodiesterase, since they have several unique properties (12).

Phosphodiesterase activity was measured by previously reported techniques (30), described briefly as follows. The usual assay mixture consisted of 10 μg of pyruvate kinase, 5 μg of myokinase, 25 mM ammonium acetate, 3 mM magnesium chloride, 0.26 mM phosphoenolpyruvate, 15 mM dithiothreitol, 0.1 mM EDTA, 0.01% bovine serum albumin, 0.1 nM ATP, varying concentrations of cyclic AMP and inhibitors or activator, and the tissue sample in a total volume of 150 μl of glycylglycine buffer, 50 mM, pH 8.0. This mixture was incubated for various times at 37° with gentle shaking. Immediately at the end of the incubation period and without terminating the previous enzymatic reactions, the samples were analyzed for ATP content by the firefly luciferin-luciferase technique, the generated light being quantitated with a luminescence biometer (du Pont).

The possibility that any of these drugs affected the phosphodiesterase assay system was accounted for by incubating these agents with the 5′-AMP standards and using this standard curve to calculate the phosphodiesterase activity when assayed in the presence of the drug.

A heat-stable, nondialyzable activator of phosphodiesterase was prepared from rat brain according to Cheung (23). The material was purified up to, but not including, the ammonium sulfate step. This produced a highly active material with no detectable phosphodiesterase activity.

K_i values were determined according to Dixon (31). In this procedure the reciprocal of the reaction velocity ($1/v$) is plotted with respect to the inhibitor concentration at different concentrations of substrate. The K_i values are obtained from the point of intersection of these lines. Experiments were generally carried out with five substrate concentrations and five inhibitor concentrations. Each point on the graph represents the mean values of three to five replications at each concentration. The standard errors for each point was generally within 10% of the mean value. The line of best fit was determined by linear regression analysis, and the point of intersection of the lines was determined graphically.

RESULTS

Multiple Forms of Phosphodiesterase of Rat Cerebrum

Figure 1 shows the pattern of cyclic 3′,5′-AMP phosphodiesterase activity in the

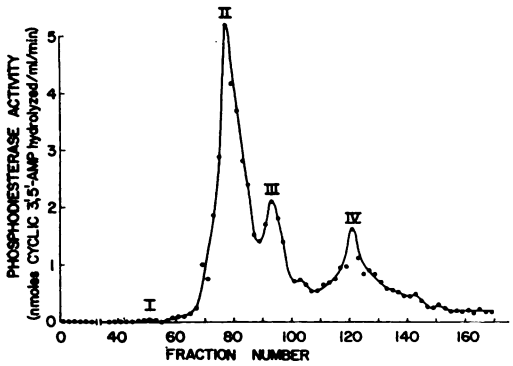

FIG. 1. *Multiple forms of phosphodiesterase of rat cerebrum*

The cerebrum was homogenized in 4 volumes of 0.32 M sucrose, sonicated, and centrifuged at 100,000 × *g* for 60 min. One milliliter of the supernatant fluid was placed on the polyacrylamide gel column. The phosphodiesterase activity of each fraction was determined as described under METHODS, using 200 μM cyclic AMP as substrate. The assays were performed in the presence of a heat-stable, nondialyzable activator prepared from rat brain (approximately 5 μg of protein).

soluble supernatant fraction of rat cerebral homogenates when measured in the presence of the activator of phosphodiesterase. Four distinct peaks of phosphodiesterase were found, labeled I–IV according to the order in which they emerged from the gel column. Peak I, although very small, was found consistently in each preparation.

Effect of Endogenous Activator on Multiple Forms of Phosphodiesterase

Table 1 shows the effect of a heat-stable, nondialyzable activator of phosphodiesterase on the different forms of phosphodiesterase. The activator had no effect on peak I or IV but increased the activity of peak II more than 10-fold. The relatively small increase in phosphodiesterase activity of peak III seen in this experiment may be accounted for by a contamination with peak II phosphodiesterase, since other experiments have shown that a highly purified peak III is totally unresponsive to the activator (8).

The possibility exists, however, that peaks I, III, and IV were relatively unresponsive to the activator because they were already maximally activated by an activator bound to the enzyme. This question was examined

TABLE 1

Effect of endogenous phosphodiesterase activator on multiple forms of phosphodiesterase of rat cerebrum

Each peak of phosphodiesterase, prepared and separated as described in the legend to Fig. 1, was assayed for phosphodiesterase activity in the absence and presence of the phosphodiesterase activator (about 5 μg of protein). Each figure represents the mean value of five determinations ± standard error.

Peak	Phosphodiesterase activity	
	No activator	With activator
	nmoles cyclic AMP hydrolyzed/ml/min	
I	23 ± 2	25 ± 1
II	603 ± 35	7918 ± 401
III	577 ± 41	1242 ± 69
IV	560 ± 28	577 ± 26

by combining the several peaks and assaying the phosphodiesterase activity. In each case the total activity was equal to the sums of the individual activities. In addition, since the activator is stable to heat whereas the phosphodiesterase is not, we boiled the preparations of peaks I, III, and IV and added this to a freshly prepared peak II. No activation of peak II was seen. These results suggest that the unresponsive peaks did not contain an activator bound to the enzyme.

Mechanism of Activation

To gain an insight into the mechanism by which the activator affected peak II phosphodiesterase, we determined the activity of the enzyme in the presence of varying concentrations of the activator and plotted the data in double-reciprocal form. The results (Fig. 2) suggest that the activator increased the maximum reaction velocity (V_{max}) several fold but did not alter the apparent Michaelis constant (K_m) (approximately 250 μM).

Effect of Inhibitors on Multiple Forms of Phosphodiesterase

Earlier reports (12, 32, 33) indicated that theophylline, papaverine, and trifluoperazine at concentrations of 1, 0.1, and 0.5 mM, respectively, have differential inhibitory

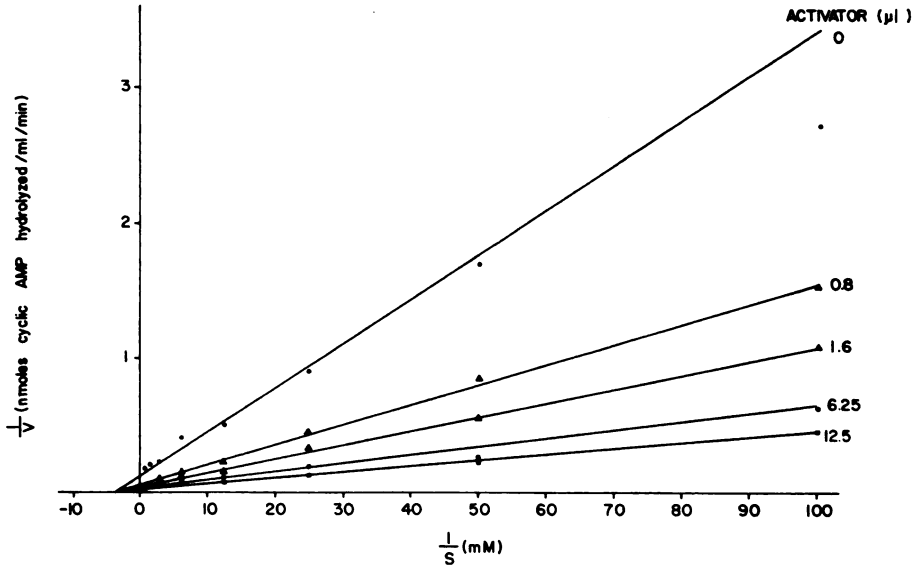

FIG. 2. *Effect of activator on cyclic AMP phosphodiesterase activity of rat cerebrum (peak II)*
The activity of phosphodiesterase in the peak II fraction of rat cerebrum was determined in the presence of varying quantities of cyclic AMP and activator as described under METHODS. One microliter of activator contained approximately 0.2 µg of protein added to 150 µl of incubation mixture. Each point represents the mean of four determinations. The K_m value was approximately 250 µM.

effects on the multiple forms of phosphodiesterase isolated from rat cerebrum. These compounds produced about the same degree of inhibition of peak I phosphodiesterase (about 30%) but elicited a markedly different inhibitory activity on the other forms of phosphodiesterase. The most notable differences were seen in peaks II and III. We therefore performed more detailed concentration-response studies of these inhibitors on peaks II and III. As can be seen (Table 2), the K_i values of theophylline, papaverine, and cyclic GMP for peak II phosphodiesterase, when measured in the absence of activator, were similar to those measured in the presence of the activator. On the other hand, the K_i value of trifluoperazine for the activated peak II was about 25 times less than that for the nonactivated peak II or for peak III (adding the activator to peak III did not alter the K_i for trifluoperazine).

Figure 3 compares the inhibitory effects of trifluoperazine, a potent phenothiazine tranquilizer, with those of two other phenothiazines which have weak psychotropic activity. Promethazine and trifluoperazine sulfoxide

TABLE 2

Differential inhibition of multiple forms of phosphodiesterase of rat cerebrum

The multiple forms of phosphodiesterase were isolated from the soluble supernatant fraction of rat cerebral homogenates as described under METHODS. Each isolated peak was assayed in the presence of varying concentrations of inhibitor and cyclic AMP. Peak II phosphodiesterase was assayed in the absence and presence of optimal amounts of the phosphodiesterase activator. The inhibitor constants (K_i values) were estimated from a plot of the reciprocal of the phosphodiesterase reaction velocity against the inhibitor concentration for at least three substrate concentrations as described by Dixon (31).

Inhibitor	K_i		
	Peak II (not activated)	Peak II (activated)	Peak III
	µM	µM	µM
Theophylline	350	350	180
Trifluoperazine	250	10	250
Papaverine	100	100	60
Cyclic GMP	20	10	25

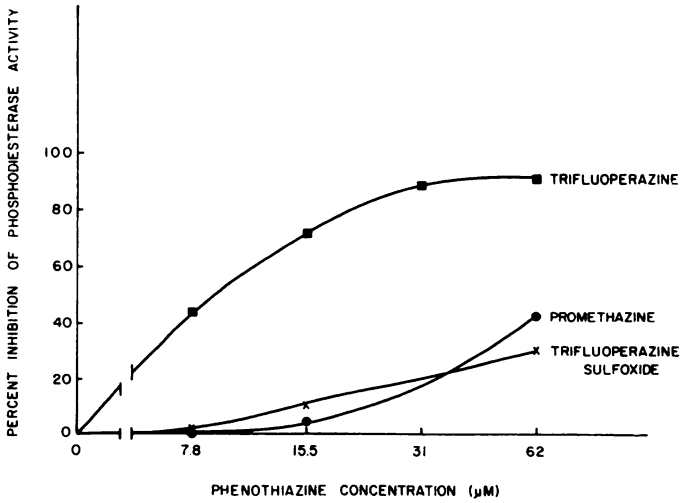

FIG. 3. *Inhibition of phosphodiesterase activity of rat cerebrum (peak II) by phenothiazine derivatives*
Samples were prepared as described in the legend to Fig. 1. The activity of peak II phosphodiesterase was analyzed in the presence of activator (about 5 μg of protein) and varying concentrations of trifluoperazine, trifluoperazine sulfoxide, or promethazine as described under METHODS. Each point represents the mean value of four determinations.

were considerably less potent inhibitors of activated peak II than was trifluoperazine (Fig. 3).

Mechanism of Inhibition of Phosphodiesterase

Theophylline. Figure 4 shows a kinetic analysis of the inhibition by theophylline of peak III and activated peak II phosphodiesterase isolated from rat cerebrum. In the concentrations used, theophylline exhibited kinetic characteristics typical of a competitive inhibitor of both peaks of phosphodiesterase; it did not alter the V_{max} but increased the apparent K_m.

Cyclic GMP. A kinetic analysis of the inhibitory effect of cyclic GMP on peak III of rat cerebrum indicates that cyclic GMP competitively inhibited the cyclic AMP phosphodiesterase (Fig. 5). Similar results were found when peak II was studied.

Papaverine. The inhibition of phosphodiesterase activity by papaverine was more complex. Figure 6 shows that concentrations of papaverine up to 50 μM inhibited peak III phosphodiesterase competitively. Higher concentrations (100–500 μM) produced noncompetitive inhibition of the enzyme (not shown).

Trifluoperazine. Kinetic analysis of the effect of trifluoperazine on peaks II and III indicated that the inhibition was neither purely competitive nor purely noncompetitive (Fig. 7). Since trifluoperazine was the most effective inhibitor of peak II phosphodiesterase, and since this peak was the one which was activated, the interaction between trifluoperazine and the activator of phosphodiesterase was studied. The experiments indicated that the degree of inhibition of peak II by trifluoperazine was influenced by the degree to which the enzyme was activated. For example, Fig. 8 shows that trifluoperazine at a concentration of 25 μM produced about 80% inhibition of the activated enzyme but no significant inhibition of the unactivated peak II phosphodiesterase. At higher concentrations trifluoperazine also inhibited the unactivated phosphodiesterase. In contrast to the results with trifluoperazine, papaverine and theophylline (not shown) inhibited the activated and unactivated peak II equally.

These results suggested that trifluoperazine was preventing the activation of peak II. To examine this further, peak II was incubated with different amounts of activator and several concentrations of trifluoperazine (Fig. 9). The results show that the

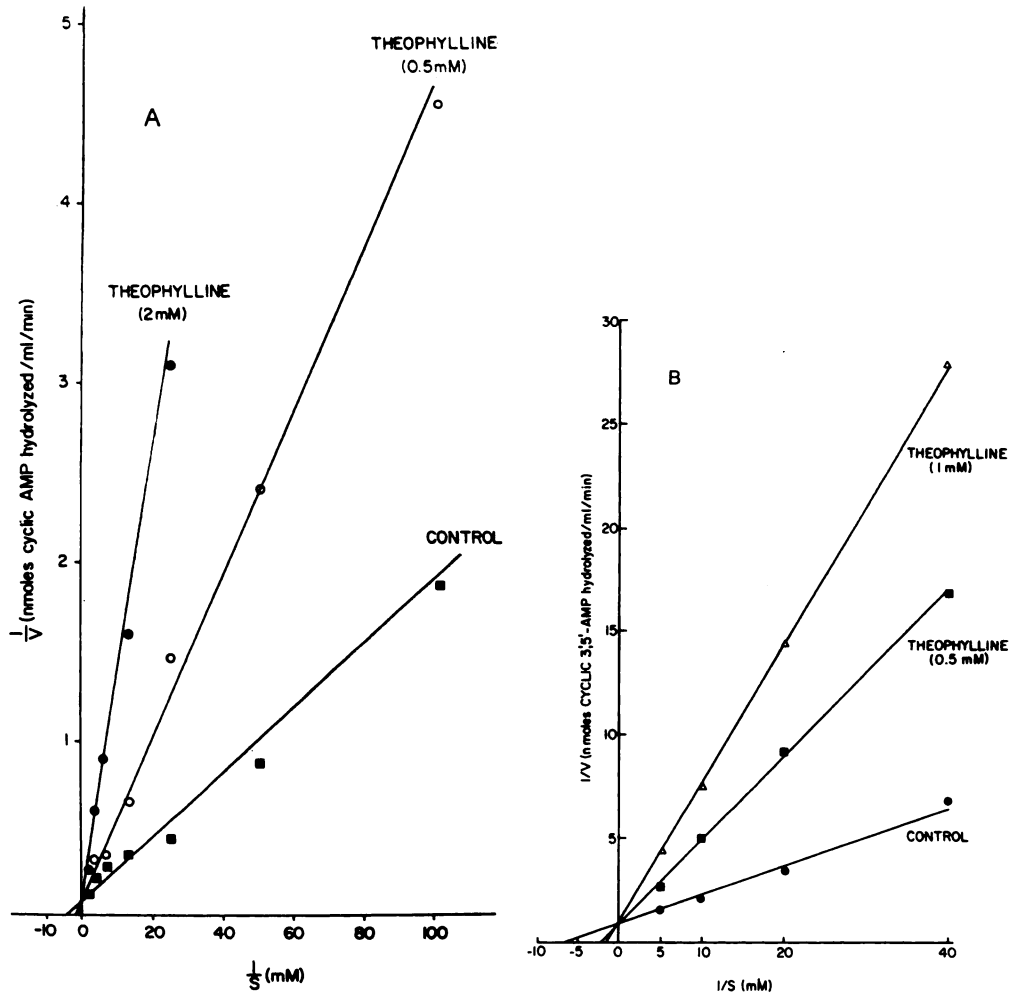

FIG. 4. *Kinetic analysis of theophylline-induced inhibition of peaks II and III phosphodiesterase of rat cerebrum*

The soluble supernatant fraction of rat cerebral homogenates was subjected to gel electrophoresis, and the activity of phosphodiesterase in the peak II and peak III fractions was determined in the presence of varying concentrations of cyclic AMP and theophylline as described under METHODS. No activator was present. Each point represents the mean of four determinations. A. Peak II. B. Peak III.

activator could antagonize the inhibition of phosphodiesterase induced by low concentrations of trifluoperazine but could not prevent the inhibition by high concentrations of the phenothiazine (Fig. 9).

DISCUSSION

Effect of Agents on Multiple Forms of Phosphodiesterase

Although several groups have now confirmed the existence of multiple forms of cyclic nucleotide phosphodiesterase (1–12), and although there is suggestive evidence that the phosphodiesterases of different tissues may be inhibited differentially by drugs (17, 20, 25–29), there is relatively little direct information on the effects of activators or inhibitors on the different forms of phosphodiesterase. Previous studies have shown that the multiple forms of phosphodiesterase isolated from rat cerebrum could be selectively activated by a heat-stable protein isolated from brain (8, 12). The present results

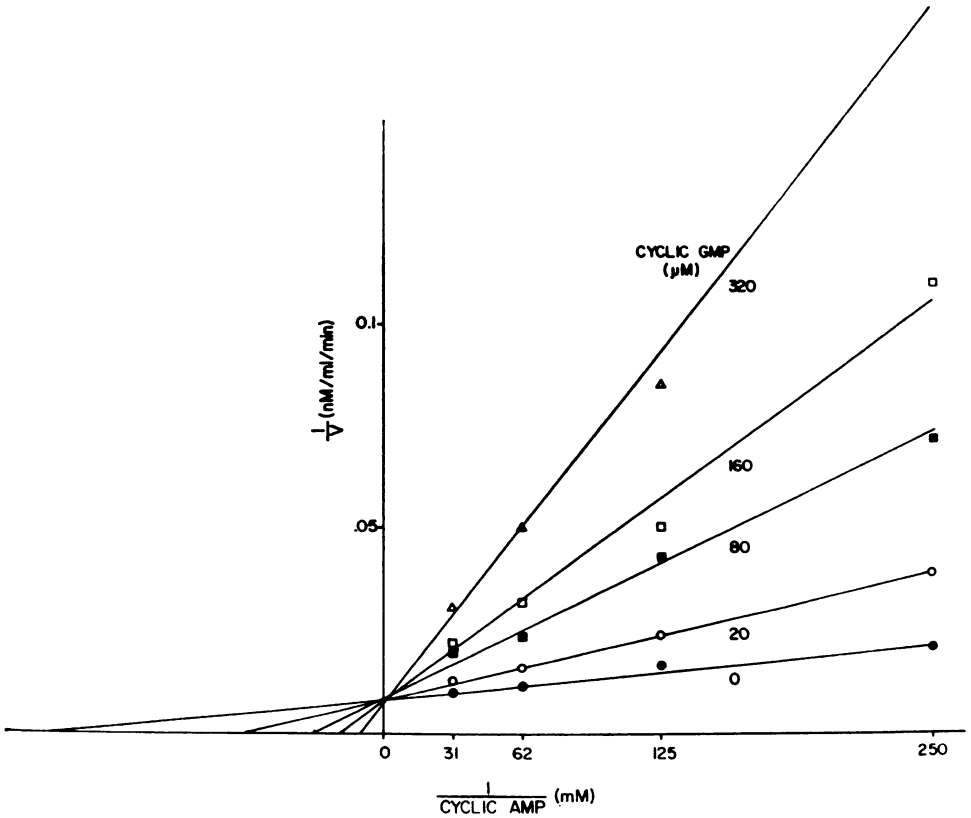

FIG. 5. *Kinetic analysis of cyclic GMP-induced inhibition of peak III phosphodiesterase of rat cerebrum*
The soluble supernatant fraction of rat cerebral homogenates was subjected to gel electrophoresis, and the activity of phosphodiesterase in the peak III fraction was determined in the presence of varying concentrations of cyclic AMP and cyclic GMP as described under METHODS.

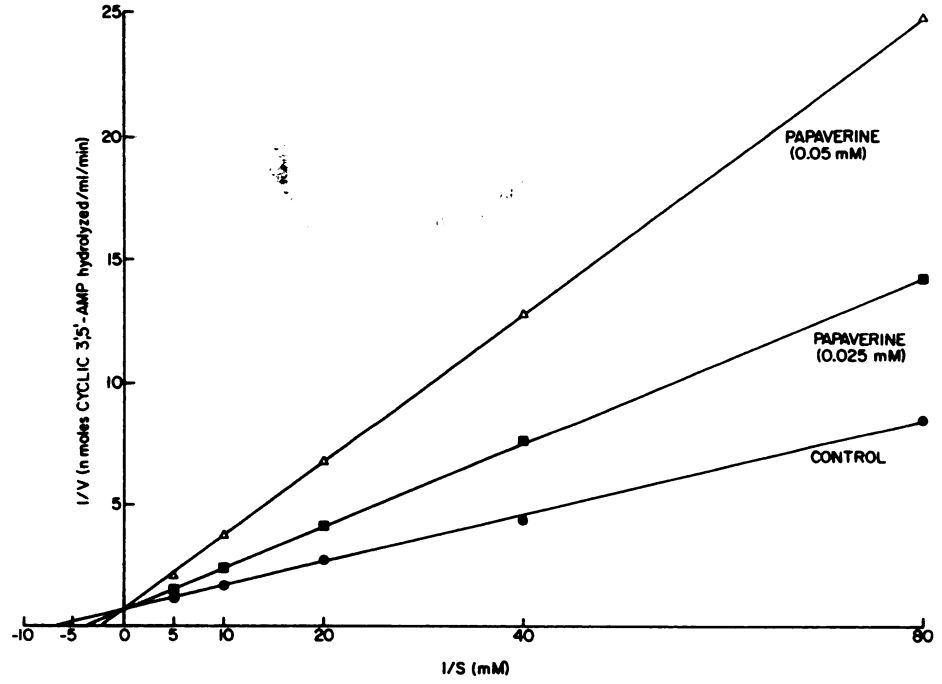

FIG. 6. *Kinetic analysis of papaverine-induced inhibition of peak III phosphodiesterase of rat cerebrum*
The soluble supernatant fraction of rat cerebral homogenates was subjected to gel electrophoresis, and the activity of phosphodiesterase in the peak III fraction was determined in the presence of varying concentrations of cyclic AMP and papaverine as described under METHODS. Each point represents the mean of four determinations.

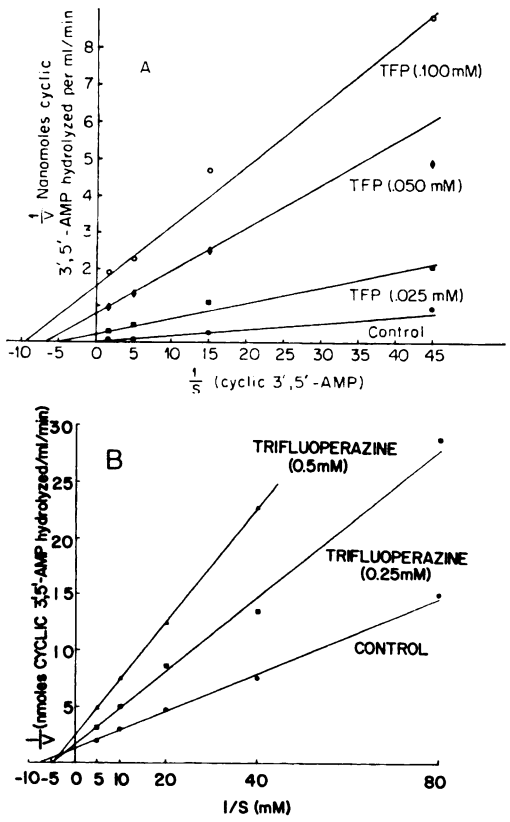

FIG. 7. *Kinetic analysis of trifluoperazine-induced inhibition of peak II and III phosphodiesterase of rat cerebrum*

The soluble supernatant fraction of rat cerebral homogenates was subjected to gel electrophoresis, and the activity of phosphodiesterase in the peak II and peak III fractions was determined in the presence of varying quantities of cyclic AMP (millimolar) and trifluoperazine (TFP) as described under METHODS. Peak II was assayed in the presence of a heat-stable activator (approximately 5 μg of protein). Each point represents the mean of four determinations. A. Peak II. B. Peak III.

show that these enzymes can also be differentially inhibited. For example, of the compounds studied, trifluoperazine was the most potent inhibitor of the activated peak II (K_i = 10 μM) and cyclic GMP was the most effective inhibitor of the unactivated peak II (K_i = 20 μM).

Mechanism of Inhibition

The various phosphodiesterase inhibitors studied differed not only in their potency but also in their mechanism of action. Theophylline inhibited the purified phosphodiesterases of rat brain, apparently by competing with cyclic AMP for the active site on the enzyme. This is in agreement with the early work of Butcher and Sutherland (13), who reported that the methylxanthines competitively inhibited the phosphodiesterase of beef heart.

The inhibitory effects of papaverine were more complex. Low concentrations of papaverine (50 μM and less) competitively inhibited the purified peaks II and III of cerebral phosphodiesterase, whereas higher concentrations inhibited the enzyme noncompetitively.

A number of authors have shown that cyclic GMP can inhibit (1, 34) or activate (35–37) cyclic AMP phosphodiesterase, depending upon the concentration of substrate and the concentration of cyclic GMP used. These reports show that cyclic GMP activated the cyclic AMP phosphodiesterase when low substrate concentrations (1–10 μM) were used but inhibited the enzyme when high concentrations of cyclic AMP were used as substrate. Using high concentrations of cyclic AMP (0.1 mM), we confirmed the finding that cyclic GMP can inhibit cyclic AMP phosphodiesterase. Our experiments did not include studies with low substrate concentrations.

The inhibitory effects of trifluoperazine on the different forms of phosphodiesterase were also complex. When peak III was studied or when peak II was studied in the absence of activator, trifluoperazine produced a mixed type of inhibition, with a K_i of approximately 250 μM. In contrast, when peak II was studied in the presence of the activator of phosphodiesterase, the K_i was only about 10 μM. This inhibitory effect of trifluoperazine on peak II could be overcome by adding more activator; a kinetic analysis of these data suggested that trifluoperazine was competitively blocking the activation of peak II phosphodiesterase. This suggests that at low concentrations, trifluoperazine prevents the activation of peak II phosphodiesterase either by competing with the activator for the same site on the enzyme or by some other mechanism, such as binding

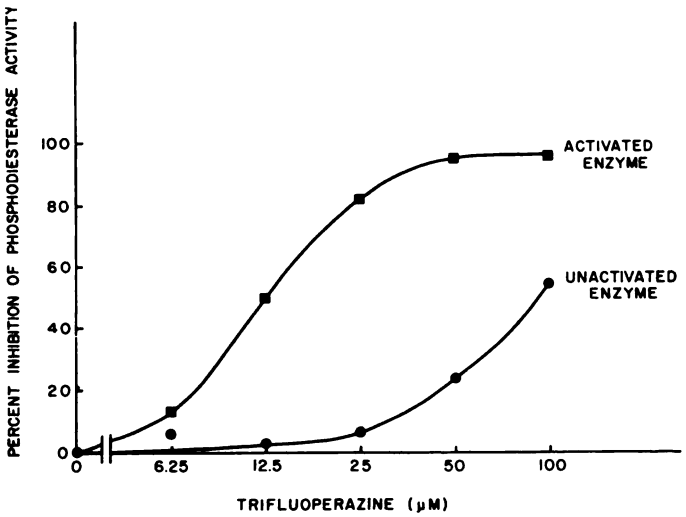

FIG. 8. *Inhibition of phosphodiesterase activity of rat cerebrum (peak II) by trifluoperazine in the presence and absence of activator*

The effect of varying concentrations of trifluoperazine on the peak II phosphodiesterase prepared from rat cerebral homogenates was determined in the absence and presence of the activator of phosphodiesterase (about 1 μg of protein) as described under METHODS. The concentration of cyclic AMP was 200 μM. Each point represents the mean of five determinations.

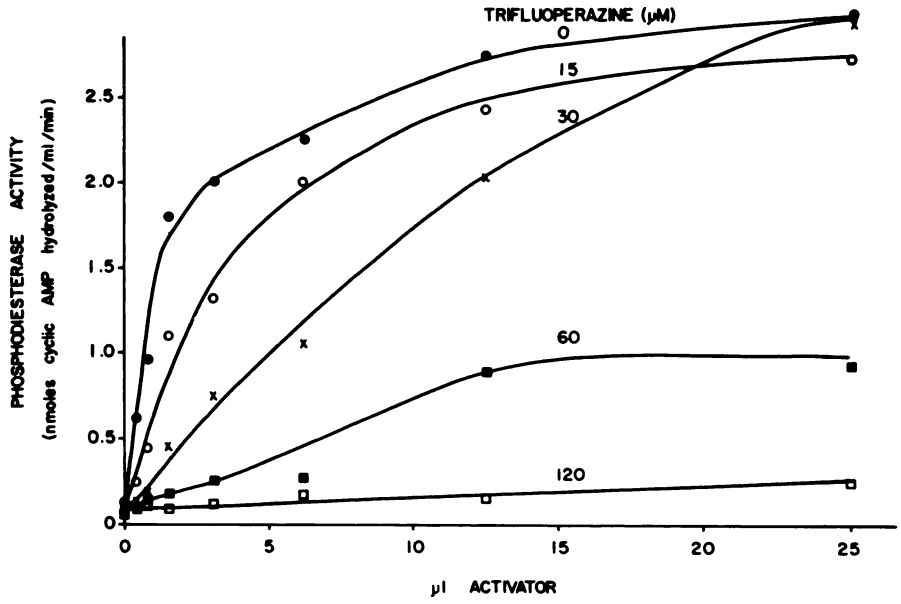

FIG. 9. *Interaction between trifluoperazine and activator on peak II phosphodiesterase of rat cerebrum*

Peak II phosphodiesterase prepared from rat cerebrum was assayed in the presence of varying concentrations of trifluoperazine and the activator of phosphodiesterase as described under METHODS. One microliter of activator contained approximately 0.2 μg of protein. The final incubation volume was 150 μl, and the concentration of cyclic AMP was 200 μM.

to the activator and thereby preventing its action.

Mechanism of Activation

The mechanism by which the activator increases phosphodiesterase activity is still an open question. It has been proposed by others (23, 38) that the activator increases the V_{max} and decreases the K_m of the phosphodiesterase. We found that the activator increases the V_{max} of peak II phosphodiesterase several fold but does not change the K_m. Although this apparent discrepancy may be due to differences in the enzyme and activator preparation used, a more likely explanation was offered by Teo, Wang, and Wang (24), who found that low concentrations of activator increase the V_{max} of phosphodiesterase but do not alter the K_m, whereas high concentrations of activator reduce the K_m as well. Since our activator fraction may have a relatively low specific activity, it is possible that we did not reach a high enough concentration of activator to affect the K_m.

Implications of the Finding that Purified Phosphodiesterase Peaks Are Differentially Affected by Drugs

The differential inhibitory effects of drugs on the phosphodiesterases of different tissues can now be understood to be the result of the unique pattern of phosphodiesterases found in each tissue (3, 5, 6, 11, 12) and the varying sensitivity of these forms of the enzyme to inhibitors. For example, the earlier finding that trifluoperazine inhibited the phosphodiesterase of rat cerebrum more than that of rat cerebellum (17) may be explained as follows. The bulk of the phosphodiesterase of rat cerebrum is peak II (Fig. 1), which, in the activated state, is very sensitive to the inhibitory effects of trifluoperazine. On the other hand, the major form of phosphodiesterase in rat cerebellum is peak III (8), which is relatively resistant to the inhibitory actions of the phenothiazine.

Besides offering an explanation for the differential effects of drugs on the phosphodiesterase activity of different tissues, these results suggest the possibility that the degree to which the phosphodiesterase activity of a tissue is affected by a given inhibitor may serve as a tool for determining the principal form of phosphodiesterase in that tissue.

We conclude that by studying the distribution of the multiple forms of cyclic nucleotide phosphodiesterase in various tissues and by determining their responses to activators and inhibitors of phosphodiesterases, it may eventually be possible to alter the concentration of the cyclic nucleotides—and thereby the function—of selective tissues by administering agents which specifically affect the phosphodiesterases of that tissue.

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REFERENCES

1. Beavo, J. A., Hardman, J. G. & Sutherland, E. W. (1970) *J. Biol. Chem.*, **245**, 5649–5655.
2. Kakiuchi, S., Yamazaki, R. & Teshima, Y. (1971) *Biochem. Biophys. Res. Commun.*, **42**, 968–974.
3. Monn, E. & Christiansen, R. O. (1971) *Science*, **173**, 540–542.
4. Thompson, W. J. & Appleman, M. M. (1971) *Biochemistry*, **10**, 311–316.
5. Thompson, W. J. & Appleman, M. M. (1971) *J. Biol. Chem.*, **246**, 3145–3150.
6. Campbell, M. T. & Oliver, I. T. (1972) *Eur. J. Biochem.*, **28**, 30–37.
7. Schroder, J. & Plagemann, G. W. (1972) *Cancer Res.*, **32**, 1082–1087.
8. Uzunov, P. & Weiss, B. (1972) *Biochim. Biophys. Acta*, **284**, 220–226.
9. Russell, T. R., Terasaki, W. L. & Appleman, M. M. (1973) *J. Biol. Chem.*, **248**, 1334–1340.
10. Weiss, B. & Strada, S. J. (1974) in *Biochemistry and Physiology of the Pineal Gland* (D. C. Klein, ed.), Spectrum, New York, in press.
11. Uzunov, P., Shein, H. M. & Weiss, B. (1973) *Science*, **180**, 304–306.
12. Uzunov, P., Shein, H. M. & Weiss, B. (1974) *Neuropharmacology*, in press.
13. Butcher, R. W. & Sutherland, E. W. (1962) *J. Biol. Chem.*, **237**, 1244–1250.
14. Markwardt, F. & Hoffmann, A. (1970) *Biochem. Pharmacol.*, **19**, 2519–2520.
15. Mandel, L. R. (1971) *Biochem. Pharmacol.*, **20**, 3413–3421.
16. Sheppard, H. & Wiggan, G. (1971) *Mol. Pharmacol.*, **7**, 111–115.

17. Uzunov, P. & Weiss, B. (1971) *Neuropharmacology*, **10**, 697–708.
18. Beer, B., Chasin, M., Clody, D. E., Vogel, J. R. & Horovitz, Z. P. (1972) *Science*, **176**, 428–430.
19. Goren, E. N. & Rosen, O. M. (1972) *Mol. Pharmacol.*, **8**, 380–384.
20. 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) *J. Pharm. Sci.*, **61**, 1556–1567.
21. Anisuzzaman, A. K. M., Lake, W. C. & Whistler, R. L. (1973) *Biochemistry*, **12**, 2041–2045.
22. Harris, D. N., Chasin, M., Phillips, M. B., Goldenberg, H., Samaniego, S. & Hess, S. M. (1973) *Biochem. Pharmacol.*, **22**, 221–228.
23. Cheung, W. Y. (1971) *J. Biol. Chem.*, **246**, 2859–2869.
24. Teo, T. S., Wang, T. H. & Wang, J. H. (1973) *J. Biol. Chem.*, **248**, 588–595.
25. Vernikos-Danellis, J. & Harris, C. G., III. (1968) *Proc. Soc. Exp. Biol. Med.*, **128**, 1016–1021.
26. Chasin, M., Harris, D. N., Phillips, M. B. & Hess, S. M. (1972) *Biochem. Pharmacol.*, **21**, 2443–2450.
27. Pichard, A. L., Hanoune, J. & Kaplan, J. C. (1972) *Biochim. Biophys. Acta*, **279**, 217–220.
28. Amer. M. S. & McKinney, G. (1973) *Life Sci.*, **13**, 753–767.
29. Kukovetz, W. R. & Poch, G. (1970) *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **267**, 189–194.
30. Weiss, B., Lehne, R. & Strada, S. J. (1972) *Anal. Biochem.*, **45**, 222–235.
31. Dixon, M. (1953) *Biochem. J.*, **55**, 170–171.
32. Fertel, R., Uzunov, P. & Weiss, B. (1973) *Fed. Proc.*, **32**, 679.
33. Weiss, B. (1974) in *Frontiers in Catecholamine Research* (E. Usdin and Snyder, eds.), pp. 327–333. Pergamon Press, New York.
34. Rosen, O. M. (1970) *Arch. Biochem. Biophys.*, **139**, 447–449.
35. Beavo, J. A., Hardman, J. G. & Sutherland, E. W. (1971) *J. Biol. Chem.*, **246**, 3841–3846.
36. Klotz, U. & Stock, K. (1972) *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **274**, 54–62.
37. Franks, D. J. & MacManus, J. P. (1972) *Biochem. Biophys. Res. Commun.*, **42**, 844–849.
38. Goren, E. N. & Rosen, O. M. (1971) *Arch. Biochem. Biophys.*, **142**, 720–723.