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Selective Inhibition of Two Soluble Adenosine Cyclic 3′,5′-Phosphate Phosphodiesterases Partially Purified from Calf Liver†

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ABSTRACT: "Low K_m" cAMP phosphodiesterase and cGMP-stimulated cyclic nucleotide phosphodiesterase activities were partially purified from calf liver supernatant by chromatography on DEAE-cellulose and DEAE-Sepharose and ammonium sulfate precipitation. The low K_m phosphodiesterase was not retained on N^6-$H_2N(CH_2)_2$-cAMP-agarose and could be separated from the cGMP-stimulated phosphodiesterase which was absorbed by this matrix. From the proteins that did not bind, two distinct low K_m cAMP phosphodiesterases were separated on Ultrogel AcA 34. One form (fraction C) hydrolyzed cAMP with an apparent K_m of $\sim$0.5 μM and was very sensitive to inhibition by cGMP. Lineweaver-Burk plots of cAMP hydrolysis by a second form (fraction B) were nonlinear, with an apparent low K_m component of $\sim$2 μM. This form was rather insensitive to inhibition by cGMP. With both fractions, hydrolysis of cAMP relative to cGMP was much greater at low ($\sim$1 μM) than at high ($\sim$100 μM) substrate concentrations. Maximal velocities for cAMP and cGMP were similar. From sedimentation equilibrium, the apparent weight-average molecular weight of fraction B was estimated as 174 000, and that of fraction C was 85 000. Another fraction (A) of cAMP phosphodiesterase eluted at the void volume of the AcA 34 column. On the basis of the relative affinities for cAMP and cGMP and inhibition by cGMP, fraction A is most likely an aggregated form of fraction B. No apparent interconversion of fractions A, B, or C was observed on high-performance liquid chromatography. Fractions B and C differed in their sensitivity to phosphodiesterase inhibitors as well as in other characteristics. The order of potency for inhibition of fraction B was RO 20-1724 (IC_{50}, 2.2 μM) > papaverine > isobutylmethylxanthine (IBMX) > cilostamide > theophylline > cGMP. The order for fraction C was cilostamide (IC_{50}, 0.03 μM) > cGMP (IC_{50}, 0.75 μM) > papaverine > IBMX > theophylline > RO 20-1724. The use of specific inhibitors may facilitate understanding the role of specific phosphodiesterases in the regulation of intracellular cAMP content.

Multiple forms of cyclic nucleotide phosphodiesteraes (EC 3.4.1.17) with distinct physcial, catalytic, and regulatory properties have been described in mammalian tissues [for a review, see Wells & Hardman (1977), Strada & Thompson (1978), and Appleman et al. (1973)]. One form, referred to as "low K_m" cAMP phosphodiesterase, has been purified from dog kidney (Thompson et al., 1979) and from bovine lung (Moore & Schroedter, 1982). Particulate fractions of several mammalian tissues also contain low K_m cAMP phosphodiesterases, one of which is known as insulin-stimulated cAMP phosphodiesterase (Weber & Appleman, 1982; Marchmont & Houslay, 1980; Houslay & Marchmont, 1981; Elks et al., 1983).

The different forms of cyclic nucleotide phosphodiesterases can be more or less selectively inhibited by certain inhibitors (Hidaka et al., 1979; Kramer et al., 1977; Chasin & Harris, 1976; Weiss & Hait, 1977; Kuo et al., 1978; Helfman & Kuo, 1982; Hidaka & Endo, 1983), as might be expected from their distinct properties. The calmodulin-activated phosphodiesterase is inhibited by trifluoperazine (Weiss & Hait, 1977) or W7 (Hidaka & Endo, 1983), drugs that presumably bind to calmodulin. It has recently been reported that this enzyme is directly and selectively inhibited by compound TCV-3B (Hidaka & Endo, 1983) and the platelet calmodulin-insensitive cGMP phosphodiesterase by MY 5445 (Hidaka & Endo, 1983). A low K_m cAMP phosphodiesterase was selectively inhibited by cilostamide (Hidaka et al., 1979) and a cCMP phosphodiesterase by Sch 15280 (Kuo et al., 1978).

We describe here characteristics of two forms of low K_m cAMP phosphodiesterase, partially purified from calf liver, with distinct hydrodynamic properties and different sensitivities to phosphodiesterase inhibitors. One form, with an apparent weight-average molecular weight (M_w^{app}) of $\sim$85 000 and an apparent K_m for cAMP of $\sim$0.5 μM, was selectively inhibited by cilostamide and was also very sensitive to inhibition by cGMP. In this regard, this enzyme was analogous to a recently

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described cAMP phosphodiesterase activity found in rat adipocytes (Weber & Appleman, 1982) and rat liver (Whitson & Appleman, 1982). A second low K_m form, with an apparent K_m for cAMP of ~2.0 μM and an M_w^{app} of 174 000, was selectively inhibited by RO 20-1724 but was relatively insensitive to inhibition by cGMP.

Experimental Procedures

Materials. [8,5-^{3}H]cGMP (35.6 Ci/mmol) and [2,8-^{3}H]cAMP (36.4 Ci/mmol), purchased from New England Nuclear, were purified by thin-layer chromatography on cellulose with 2:5 (v/v) 0.5 M ammonium acetate:ethanol and columns (0.5 × 3 cm) of DEAE-Sephadex A-25 (Pharmacia). Dipyridamole and Ro 20-1724 were gifts from Boehringer Ingelheim, NIADDK, and Dr. M. Lin, NIH. Isobutylmethylxanthine (IBMX)[1] was purchased from Aldrich; papaverine and theophylline were from Sigma. Cilostamide and MY 5445 were provided by the Laboratories of Medical Chemistry, Otsuka Pharmaceutical Co., Ltd., Japan, and Mitsubishi Yuka Co., Ltd., Japan, respectively. IBMX, papaverine, theophylline, and RO 20-1724 were dissolved in water, dipyridamole in methyl alcohol, cilostamide in ethyl alcohol, and MY 5445 in Me_2SO. Methyl alcohol, ethyl alcohol, or Me_2SO at concentrations of 1% (present in assays) had no effect on enzyme activities. *Crotalus atrox* venom, cGMP, cAMP, 5′-GMP, 5′-AMP, BSA, PMSF, and pepstatin A were purchased from Sigma; Ultrogel AcA 34 was from LKB; Hepes (Ultrol) was from Calbiochem-Behring; Leupeptin was from Boehringer-Mannheim; DTT was from Bethesda Research Laboratories.

Preparation of Enzymes. A fraction containing both low K_m cAMP phosphodiesterase and cGMP-stimulated phosphodiesterase activities was partially purified from calf liver by conventional procedures, including chromatography on DEAE-cellulose and DEAE-Sepharose and ammonium sulfate precipitation. The cGMP-stimulated phosphodiesterase was further purified to apparent homogeneity by selective absorption to and elution from N^6-$H_2N(CH_2)_2$-cAMP–agarose (Yamamoto et al., 1983a). Proteins that did not bind to N^6-$H_2N(CH_2)_2$-cAMP–agarose were chromatographed on Ultrogel AcA 34 to separate two forms of low K_m cAMP phosphodiesterase as described under Results.

Phosphodiesterase Assay. Phosphodiesterase activity was assayed as described by Manganiello & Vaughan (1973) and modified by Yamamoto et al. (1983a). Activities reported represent verified initial rates of hydrolysis with <20% of substrate hydrolyzed. Data reported are means of values for duplicate assays in representative experiments, each of which was replicated 2 or more times.

Protein Determination. Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method (Bradford, 1976) with bovine serum albumin as the standard.

Results

The final step in the purification of cGMP-stimulated cyclic nucleotide phosphodiesterase from calf liver supernatant was affinity chromatography using N^6-$H_2N(CH_2)_2$-cAMP–agarose (Yamamoto et al., 1983a). The fraction not retained by that matrix was enriched in cAMP phosphodiesterase and depleted of cGMP and cGMP-stimulated phosphodiesterase activities (Table I). Chromatography of this fraction on Ultrogel AcA 34 partially separated at least three major types of phosphodiesterase which differed in molecular size (Figure 1). These were designated as fractions A, B, and C according to their order of elution. On occasion, some cGMP-stimulated phosphodiesterase activity was present in fraction B. This

Table I: Separation of cAMP Phosphodiesterase Activity from cGMP-Stimulated Activity on N^6-$H_2N(CH_2)_2$-cAMP-Agarose[a]

		total phosphodiesterase activity (nmol/min)		
expt	fraction	[^{3}H]cAMP	[^{3}H]cAMP plus cGMP	[^{3}H]cGMP
1	applied	690	2880	1550
	not retained	390	320	95
2	applied	477	3312	1786
	not retained	311	314	114
	AcA fraction B			
	applied	40	67	ND[b]
	not retained	25	20	

[a] A partially purified (see Experimental Procedures) fraction from calf liver (experiment 1, ~400 mg of protein in 250 mL; experiment 2, ~700 mg of protein in 490 mL) in buffer A (50 mM Hepes, pH 7.5, 1 mM EDTA, 1 mM NaN_3, 10 mM NaF, 1 μM pepstatin A, 1 μM leupeptin, 1 μg/mL soybean trypsin inhibitor, 0.4 mM PMSF, and 1 mM DTT) containing 10 mM NaCl and adjusted to pH 6.0 was applied to a column (2 × 2.5 cm) of N^6-$H_2N(CH_2)_2$-cAMP-agarose. Samples of the material applied and of that which did not bind to the column were adjusted to pH 7.5 and assayed for phosphodiesterase activity with 0.5 μM [^{3}H]cAMP, with 0.5 μM [^{3}H]cAMP plus 1 μM cGMP, and with 0.5 μM [^{3}H]-cGMP. In experiment 2, the material not retained was concentrated, applied to, and eluted from AcA 34 (as described in Figure 1). A fraction analogous to fraction B in Figure 1 (114 mg of protein in 44 mL) which contained residual cGMP-stimulated activity was concentrated, adjusted to pH 6, and applied to N^6-$H_2N(CH_2)_2$-cAMP-agarose as described above. [b] ND, not determined.

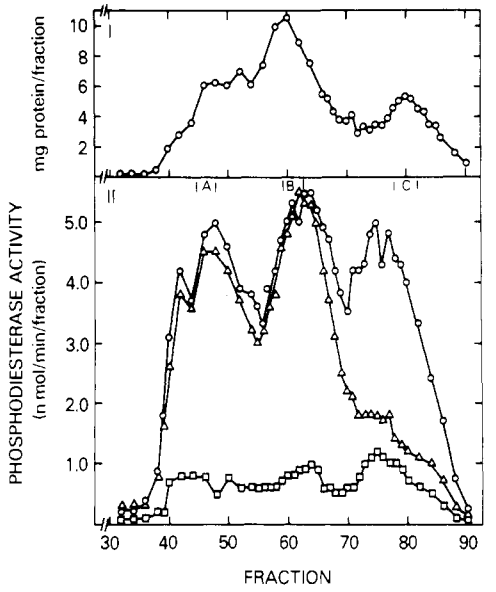

FIGURE 1: Chromatography on AcA 34 of phosphodiesterase activities which did not bind to N^6-$H_2N(CH_2)_2$-cAMP–agarose. Material which did not bind to N^6-$H_2N(CH_2)_2$-cAMP–agarose (282 mg of protein in 55 mL) was applied to a column (5 × 100 cm) of Ultrogel AcA 34 equilibrated and eluted (flow rate 67 mL/h) with buffer A containing 100 mM NaCl. Portions of fractions (16.7 mL) were analyzed for protein (top panel) and assayed for phosphodiesterase activity (bottom panel) with 0.5 μM [^{3}H]cAMP without (○) and with (△) 1 μM cGMP and with 0.5 μM [^{3}H]cGMP (□). Of the phosphodiesterase activity applied, >85% was recovered under all assay conditions. Fractions A, B, and C, designated as | |, were combined and concentrated.

[1] Abbreviations: IBMX, isobutylmethylxanthine; PMSF, phenylmethanesulfonyl fluoride; Me_2SO, dimethyl sulfoxide; BSA, bovine serum albumin; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; DEAE, diethylaminoethyl; HPLC, high-performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid.

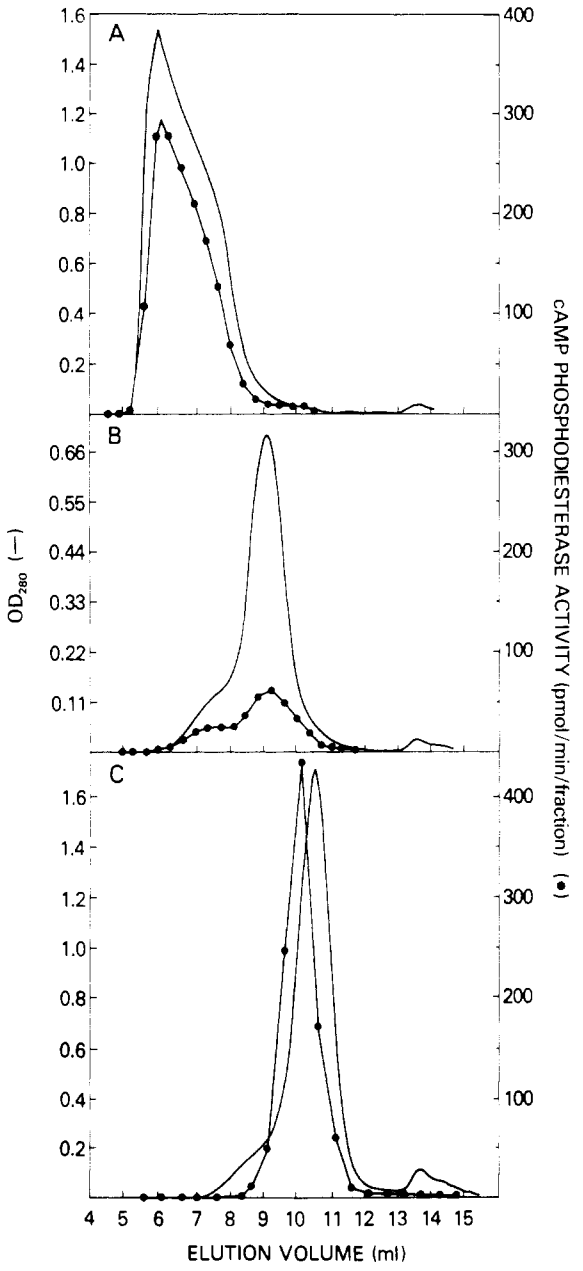

FIGURE 2: HPLC of fractions A, B, and C. After chromatography on AcA 34, fractions analogous to fractions A, B, and C in Figure 1 were combined and concentrated; fraction B was rechromatographed on N^6-$H_2N(CH_2)_2$-cAMP–agarose to remove residual cGMP-stimulated phosphodiesterase activity. Portions of each fraction [0.250 mL containing ~3.4 mg of protein (A), ~2.7 mg of protein (B), or ~1.2 mg of protein (C) after dialysis against buffer (50 mM Hepes, pH 7.5, 1 mM EDTA, 100 mM NaCl, 2 μM leupeptin, 1 μM pepstatin A, 10 mM NaF, and 100 μg/mL bovine serum albumin)] were applied to a TSK 4000 column (Toyo Soda, Japan) and eluted in the same buffer. HPLC was performed by using a Beckman Model 110 A pump; OD_{280} was monitored by using a Beckman 160 detector. Fractions were collected and assayed for phosphodiesterase activity with 0.5 μM [^{3}H]cAMP. Of the activity applied, >90% was recovered from all three fractions.

residual cGMP-stimulated activity could be separated from the cAMP phosphodiesterase activity in fraction B by rechromatography on N^6-$H_2N(CH_2)_2$-cAMP–agarose (Table I, experiment 2). When assayed with 0.5 μM substrate, all fractions hydrolyzed cAMP more rapidly than cGMP (Figure 1); cGMP (1 μM) had little effect or inhibited cAMP hydrolysis slightly in fractions A and B (Figure 1, Table I, experiment 2) but markedly inhibited cAMP hydrolysis by fraction C (Figure 1).

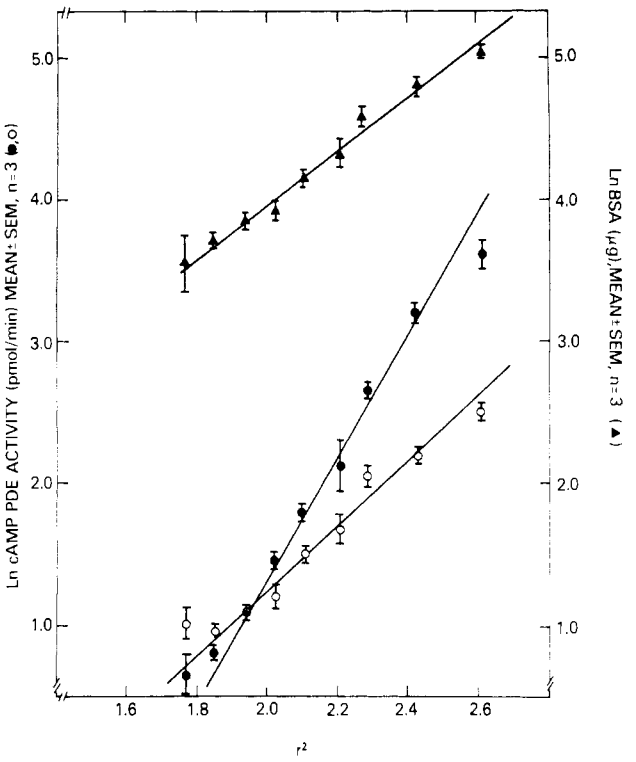

FIGURE 3: Sedimentation equilibrium of fractions B and C. Portions of fractions B and C in 100 μL of buffer A containing bovine serum albumin, 7.5 mg/mL, were centrifuged for 44 h at 20 900 rpm in a table-top Beckman Spinco airfuge in a cold room (5 °C). Rotational velocity was determined with a stroboscope. After centrifugation, tubes were placed in a vertical position, and successive 10-μL fractions were withdrawn slowly from the meniscus with a 10-μL Hamilton syringe; 94–98 μL was recovered from each tube. Samples of each fraction were diluted and assayed for protein (▲) and phosphodiesterase activity (●, ○) with 0.5 μM [^{3}H]cAMP. Of the activity applied, from fraction B (●) (155 nmol/min) recovery was 81 ± 1.2% (mean ± SEM, n = 3), and from fraction C (○) (6.3 nmol/min), recovery was 85.5 ± 2% (mean ± SEM, n = 3). The relationship between the volume and radial distance from the center of rotation was determined as described (Bothwell et al., 1978; Pollet et al., 1979) for the Beckman Spinco airfuge. Apparent weight-average molecular weights (M_w^{app}) were calculated according to the equation $M_w^{app} = [2RT/\omega^2(1-\bar{\nu}\rho)](\mathrm{d} \ln A/\mathrm{d}r^2)$ where A is the activity at position r in the cell, r is the radial distance from the center of rotation in centimeters, ω^2 is the angular velocity, $\bar{\nu}$ is the partial specific volume (taken to be 0.73), ρ is the density (measured 1.01 g/mL), R is the gas constant, and T is the absolute temperature. From these data, M_w^{app} for BSA was 68 000; for fraction B, M_w^{app} was 174 000; for fraction C, M_w^{app} was 85 000.

Fractions A, B, and C were concentrated, and a portion of each was subjected to high-performance liquid chromatography (HPLC) (Figure 2). The individual fractions eluted as predicted from chromatography of the original mixture on Ultrogel AcA 34. Fraction A eluted near the void volume of the TSK 4000 column; the apparent molecular size of fraction B was greater than that of fraction C (Figure 2). There was no evidence for extensive interconversion of any of the three fractions during HPLC (Figure 2). From sedimentation equilibrium, the M_w^{app} of fraction B based on activity measurements was 174 000, and that of fraction C was 85 000 (Figure 3) (fraction A, which eluted near the void volume of both AcA 34 and TSK 4000, was not analyzed with this technique). Fraction B exhibited a higher sedimentation coefficient than fraction C on sucrose density gradient centrifugation; by this technique, there was no evidence for aggregation of fraction C or dissociation of fraction B (data not shown).

Lineweaver–Burk plots of cAMP hydrolysis by fractions A and B were nonlinear with apparent low K_m components of

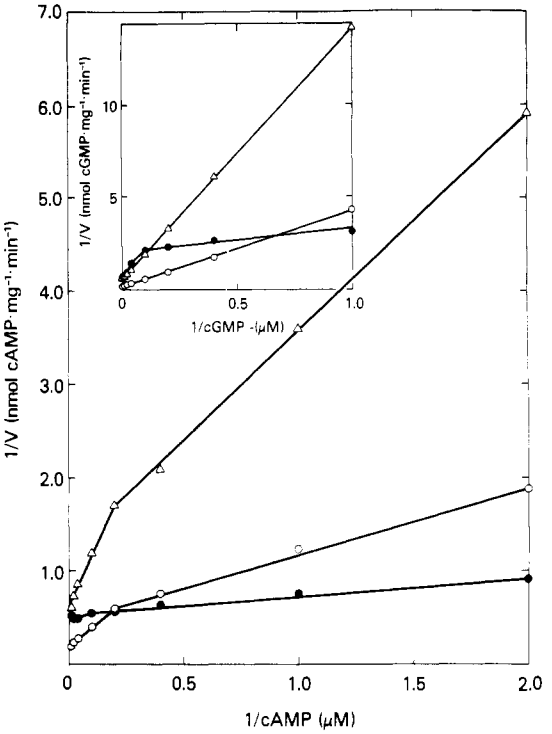

FIGURE 4: Lineweaver–Burk plots for hydrolysis of cAMP and cGMP. Portions of fractions A (○), B (△), and C (●) were assayed with the indicated concentrations of cAMP or cGMP (insert).

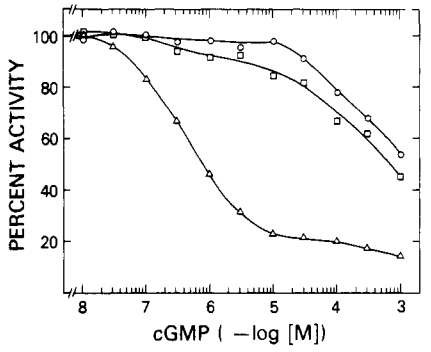

FIGURE 5: Effect of cGMP on hydrolysis of cAMP. Samples of fractions A (○), B (□), and C (△) were assayed with 1.0 μM [^{3}H]cAMP with or without the indicated concentrations of cGMP. Activities are reported relative to those in the absence of cGMP (=100%) (0.92, 0.55, and 0.62 nmol min^{-1} mg^{-1} for A, B, and C, respectively).

~2 μM (Figure 4). Fraction C hydrolyzed cAMP with a lower apparent K_m of ~0.5 μM; Lineweaver–Burk plots were linear or exhibited slight downward curvature at cAMP concentrations >100 μM (Figure 4). For all three fractions, hydrolysis of cAMP relative to cGMP was much greater at lower (1.0 μM) than at higher (100 μM) substrate concentrations (Figures 1 and 4). With 1.0 μM substrate, the ratio of hydrolysis of cAMP/cGMP was 3.5–4 for all fractions; at 100 μM substrate, the ratio was 1.2–1.3 for fractions A and B and ~2 for fraction C (Figure 4). The V_{max} for hydrolysis of cAMP and cGMP was similar for each fraction (Figure 4). Fractions A and B hydrolyzed cGMP with an apparent K_m of ~16 μM; Lineweaver–Burk plots for hydrolysis of cGMP by fraction C were nonlinear with an apparent low K_m component of ~0.7 μM (Figure 4, insert).

cGMP markedly inhibited hydrolysis of 1.0 μM [^{3}H]cAMP by fraction C (IC_{50}, ~0.75 μM) (Figure 5); inhibition by cGMP of fractions A (IC_{50}, ~700 μM) and B (IC_{50}, ~1000 μM) was much less (Figure 5). Inhibition by cGMP was less at higher concentrations of cAMP.

Table II: Effect of Inhibitors on cAMP Hydrolysis by Two Forms of Low K_m cAMP Phosphodiesterase from Calf Liver Supernatant[a]

inhibitor	phosphodiesterase fraction IC_{50} (μM) for B	C
IBMX	7.5 ± 0.4[b]	2.6 ± 0.1
papaverine	2.7 ± 0.1	1.0 ± 0.2
RO 20-1724	2.2 ± 0.4	267 ± 17
cilostamide	>100	0.03 ± 0.01
theophylline	343 ± 23	120 ± 17
MY 5445	>100	>100

[a] Samples of fraction B (0.36 ± 0.02 nmol min^{-1} mg^{-1}; mean ± SEM, n = 4) and fraction C (0.53 ± 0.02 nmol min^{-1} mg^{-1}; mean ± SEM, n = 4) were assayed with 0.5 μM [^{3}H]cAMP without or with several concentrations (at least six to eight) of each inhibitor. IC_{50} values were determined graphically as shown in Figure 6. [b] Mean ± SEM, n = 3 or 4.

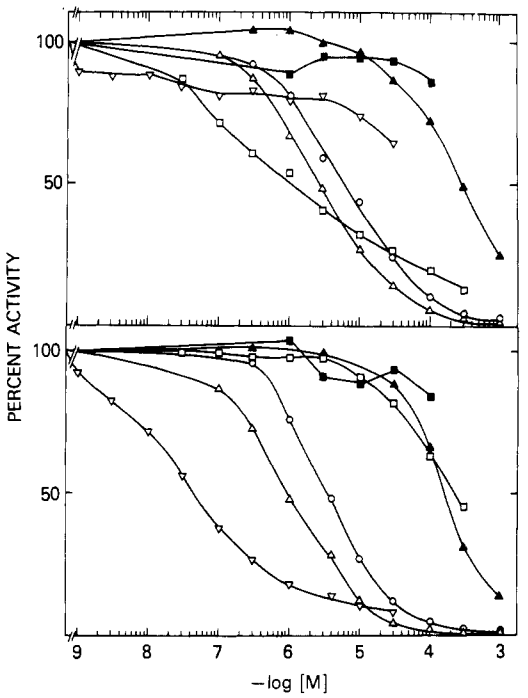

FIGURE 6: Effects of phosphodiesterase inhibitors on cAMP hydrolysis by fraction B (top panel) and fraction C (bottom panel). Samples of fractions B and C were assayed with 0.5 μM [^{3}H]cAMP with the indicated concentrations of RO 20-1724 (□), papaverine (△), IBMX (○), cilostamide (▽), theophylline (▲), and MY 5445 (■). Activities are reported relative to that in the absence of inhibitors [for fraction B, 0.41 nmol min^{-1} (mg of protein)$^{-1}$, for fraction C, 0.50 nmol min^{-1} (mg of protein)$^{-1}$ = 100%].

For the inhibitors tested, the order of potency for inhibition of cAMP (0.5 μM) hydrolysis for fraction B was RO 20-1724 > papaverine > IBMX (Figure 6, Table II). MY 5445, theophylline, and cilostamide were not effective inhibitors of fraction B (Figure 6, Table II). The order of potency for inhibition of fraction C was cilostamide > papaverine > IBMX > theophylline (Figure 6, Table II). Thus, cilostamide and cGMP were relatively specific inhibitors for fraction C and RO 20-1724 for fraction B (Figure 6, Table II). Structures of the inhibitors are shown in Figure 7.

Discussion

Rat liver supernatant contains several types of cyclic nucleotide phosphodiesterases including calmodulin-sensitive and -insensitive cGMP phosphodiesterases (Strewler et al., 1983), a cGMP-stimulated phosphodiesterase (Moss et al., 1977; Vaughan et al., 1981; Manganiello et al., 1983), and a low

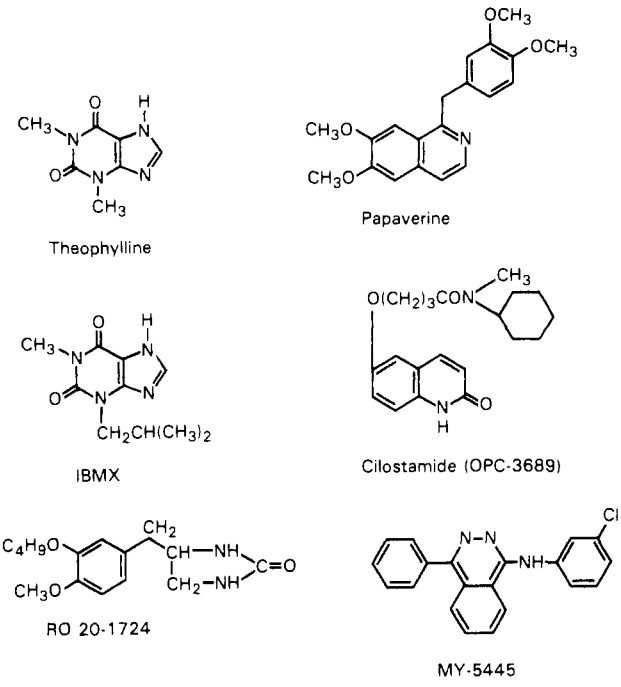

FIGURE 7: Structure of inhibitors.

K_m cAMP phosphodiesterase (Russell et al., 1973). As described here, calf liver supernatant contained at least two types of low K_m cAMP phosphodiesterase activities which were not completely separated from the cGMP-stimulated phosphodiesterase by conventional techniques. After adsorption of the cGMP-stimulated enzyme to N^6-$H_2N(CH_2)_2$-cAMP–agarose, two distinct low K_m cAMP phosphodiesterases, which were not retained by cAMP–agarose, were separated by chromatography on AcA 34. One form, with an apparent K_m of 0.5 μM for cAMP, exhibited an M_w^{app} of 85 000. This form, which also hydrolyzed cGMP (K_m ~0.7 μM), was readily inhibited by cGMP and cilostamide. This activity is apparently analogous to the low K_m cAMP phosphodiesterase described by Appleman and co-workers (Russell et al., 1973) and to an enzyme from rat platelets that was selectively inhibited by cilostamide (Hidaka et al., 1979). A second form (fraction B), with a slightly higher K_m (~2.0 μM) for cAMP and an M_w^{app} of 174 000, was not readily inhibited by either cGMP or cilostamide but was much more sensitive to RO 20-1724. At low (i.e., 1 μM) substrate concentrations, the rate of cAMP hydrolysis in both fractions was severalfold greater than that for cGMP hydrolysis; apparent maximal velocities, however, were similar for both substrates. Whitson & Appleman (1982) also separated two types of low K_m cAMP phosphodiesterases from a rat liver particulate fraction, only one of which was inhibited by cGMP.

The second low K_m cAMP phosphodiesterase (i.e., fraction B) has not previously been described in any detail, presumably because it can be eluted from ion-exchange and gel filtration columns in close association with the cGMP-stimulated phosphodiesterase, from which it was separated by chromatography on N^6-$H_2N(CH_2)_2$-cAMP–agarose. On the basis of the relative affinities of cAMP and cGMP and the characteristics of the inhibition of cAMP hydrolysis by cGMP, fraction A may represent an aggregated form of the RO 20-1724 sensitive enzyme (fraction B). No apparent interconversion was observed, however, on HPLC of isolated fractions A and B.

The inhibitors and cGMP had very different effects on the low K_m cAMP phosphodiesterases and the cGMP-stimulated phosphodiesterase that we purified from calf liver (Yamamoto et al., 1983a). As might be expected from earlier studies (Appleman et al., 1973; Moss et al., 1977), hydrolysis of appropriate concentrations of cAMP by the purified calf liver enzyme was markedly enhanced by cGMP (Yamamoto et al., 1983a). In contrast, the low K_m cAMP enzymes were either little affected (fractions A and B) or markedly inhibited (fraction C) by cGMP. The purified cGMP-stimulated enzyme was relatively insensitive to inhibition by RO 20-1724 and, although inhibited by cilostamide, was much less sensitive to cilostamide than was fraction C. At low substrate concentrations, IBMX, papaverine, and dipyridamole but not cilostamide altered cooperative interactions and increased the activity of the cGMP-stimulated enzyme (Yamamoto et al., 1983b).

Although the two types of low K_m cAMP phosphodiesterases have distinct physical and catalytic activities, and different sensitivities to phosphodiesterase inhibitors, it remains to be determined whether they represent discrete gene products or modified forms of another enzyme(s). The results of these studies emphasize problems that can arise from using a single phosphodiesterase inhibitor to prevent cyclic nucleotide degradation in cells or tissues without measuring the cyclic nucleotide content or knowing the characteristics of phosphodiesterase(s) present. With regard to the latter, it may be possible in certain cells to use these inhibitors to evaluate the importance of the RO 20-1724 sensitive and cilostamide-sensitive cAMP phosphodiesterases in the regulation of cAMP content.

Acknowledgments

We thank D. Marie Sherwood and Barbara L. Mihalko for excellent secretarial assistance.

Registry No. cAMP, 60-92-4; cGMP, 7665-99-8; RO 20-1724, 29925-17-5; IBMX, 28822-58-4; papaverine, 58-74-2; cilostamide, 68550-75-4; theophylline, 58-55-9; cAMP phosphodiesterase, 9036-21-9.

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Purification and Characterization of Chemotactic Methylesterase from *Bacillus subtilis*†

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ABSTRACT: By utilization of methanol evolution as an assay, a protein methylesterase from *Bacillus subtilis* has been purified. A 1200-fold purification has been achieved by CM-Bio-Gel A, hydroxylapatite, and Bio-Gel P-60 column chromatography. Gel filtration and sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicate the enzyme is a monomer of 41 000 in molecular weight. The enzyme is stabilized and activated by aqueous glycerol solutions. Methyl-accepting chemotaxis proteins (MCPs) serve as substrates for the enzyme. The enzyme requires divalent cation for activity, with maximum activity obtained at 1.1 mM Mg^{2+}. The enzyme is most active at pH 7.5 and at 28 °C. Methylesterase has an apparent K_m for methylated MCPs of about 10 nM.

Posttranslational modification of polypeptides allows proteins to be rapidly modified without the expense of new protein synthesis. In the case where the modifications are reversible, transient changes can occur, and later, the unmodified protein can be regenerated. One such modification, methyl esterification, has been shown to play an important role in bacterial and leukocyte chemotaxis (Goy et al., 1977; Goldman et al., 1982; O'Dea et al., 1978) and is associated with secretion from the adrenal medulla (Gagnon et al., 1978b), posterior pituitary (Gagnon et al., 1978a), and parotid gland (Gagnon et al., 1979).

In the case of bacterial chemotaxis, the response to an increase in the concentration of an amino acid attractant is a change in degree of methylation of certain intrinsic membrane proteins, the methyl-accepting chemotaxis proteins (MCPs) (Goy et al., 1977; Goldman et al., 1982) on certain glutamate residues (Kleene et al., 1977; Burgess-Casler, 1982). During the period when the degree of methylation of MCPs is changing, the bacteria swim smoothly and, afterward, return to their normal erratic swimming. In the Gram-negative *Escherichia coli*, the MCPs become more methylated (Goy et al., 1977); in the Gram-positive *Bacillus subtilis*, the MCPs become less methylated, with production of methanol (Goldman et al., 1982).

Our laboratory has been involved in understanding the enzymology of the process of methylation and demethylation of the MCPs. Previously, we purified and characterized two methyltransferases that catalyzed transfer of methyl groups from *S*-adenosylmethionine to MCPs. In this paper, we describe the purification and characterization of a methylesterase that removes these methyl groups, with production of methanol (Goldman et al., 1982; Toews & Adler, 1979). Stock & Koshland (1978) first reported on the existence of a methylesterase involved in bacterial chemotaxis. Although the closely related enzyme from *E. coli* was found to be 38 000 in molecular weight (Silverman & Simon, 1977), G-100 chromatography on an extract from *Salmonella typhimurium* showed the enzyme to be 100 000 in molecular weight in its native form (Stock & Koshland, 1978). Pseudorevertant studies indicated that the methylesterase interacts with the *cheZ* gene product in *E. coli* (Parkinson, 1977). Thus, the 100 000 molecular weight activity observed on a gel-filtration column may represent either a homotypic or heterotypic multimer. It has also been observed that the methylesterase has the ability to carry out a second modification on proteins distinct from demethylation, possibly deamidation (Kehry & Dahlquist, 1982). Purification of the enzyme would allow a

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