# Characterization of Soluble Uterine Cyclic Nucleotide Phosphodiesterase†

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ABSTRACT: Soluble cyclic nucleotide phosphodiesterase of rat uterus displays distinct structural and regulatory properties. Like phosphodiesterases from many mammalian sources the soluble uterine enzyme system exhibits nonlinear Lineweaver–Burk kinetics with cyclic adenosine 3′:5′-monophosphate (cAMP) as substrate (apparent $K_m$s $\simeq$ 3 and 20 $\mu$M) and linear kinetics with cyclic guanosine 3′:5′-monophosphate (cGMP) as substrate (apparent $K_m \simeq 3$ $\mu$M). Unlike most other mammalian phosphodiesterases, however, numerous separation procedures reveal only a single form of uterine phosphodiesterase which catalyzes the hydrolysis of both cAMP and cGMP. A single form of the enzyme is observed upon sucrose gradient centrifugation (7.9 S), agarose gel filtration, and DEAE-cellulose chromatography at either pH 8.0 or 6.0. Heat denaturation (50 °C) of soluble uterine phosphodiesterase causes the loss of both cAMP and cGMP hydrolytic activities at the same rate. Isoelectric focusing reveals major (p$I$ = 5.2) and minor forms (p$I$ = 5.8) of phosphodiesterase which both catalyze the hydrolysis of the two cyclic nucleotide substrates. In vivo administration of estradiol produces identical decreases in the activities of cAMP and cGMP phosphodiesterase. These results raise the possibility that the uterus contains a single form of soluble phosphodiesterase which catalyzes the hydrolysis of both cAMP and cGMP.

Regulation of cyclic nucleotide phosphodiesterase (EC 3.1.4.17) activity is an important mechanism for controlling cellular levels of cyclic nucleotides (Appleman et al., 1973; Strada & Robison, 1974; Wells & Hardman, 1977; Strada & Thompson, 1978). This regulation is complex and involves a number of different control mechanisms. Hormones (reviewed in Appleman et al., 1973; Wells & Hardman, 1977; Thompson & Strada, 1977), growth conditions of cultured cells (Anderson et al., 1973; Russell & Pastan, 1974; Strada & Pledger, 1975; Pledger et al., 1975a,b), endogenous protein activators (Cheung, 1971; Kakiuchi et al., 1973; Watterson et al., 1976; Stevens et al., 1976), and cyclic nucleotides themselves (Beavo et al., 1971; Russell et al., 1972; Mangianello & Vaughn, 1972; Thompson et al., 1973; Russell et al., 1973; Van Inwegen et al., 1977) have all been shown to affect enzyme activity. Furthermore, most mammalian tissues contain multiple forms of cyclic nucleotide phosphodiesterase with different kinetic and regulatory properties (Thompson & Appleman, 1971a,b; Appleman et al., 1973; Appleman & Terasaki, 1975; Wells & Hardman, 1977; Strada & Thompson, 1978). One implication of these findings is that the activity of different enzyme forms may be differentially regulated or that the interconversion of enzyme forms may itself be a regulatory mechanism. Because of the complexity of the cyclic nucleotide phosphodiesterase system, it is therefore necessary to define the catalytic and structural properties of the enzyme to elucidate the molecular mechanisms which control enzyme activity and the role of this enzyme system in the regulation of cellular function.

We have previously observed that the in vivo administration of estrogen decreases the activity of uterine cyclic nucleotide phosphodiesterase measured in homogenates (Stancel et al., 1975) or cytosolic preparations (Gardner et al., 1976). Developmental studies also indicated that changes in uterine growth, presumably mediated by endogenous estrogens, correlate with decreased levels of uterine cyclic nucleotide phosphodiesterase activity (Stancel et al., 1975). To investigate the biochemical basis for these decreases in phosphodiesterase activity we have initiated a study of the structural, catalytic, and regulatory properties of the uterine enzyme system.

In this report we present the results of studies dealing primarily with the structural properties of uterine phosphodiesterase. In contrast to most previous studies of mammalian phosphodiesterase (Thompson & Appleman, 1971a,b; Appleman et al., 1973; Appleman & Terasaki, 1975; Wells & Hardman, 1977; Strada & Thompson, 1978), our results suggest that the uterus contains a single form of the enzyme which catalyzes the hydrolysis of both cAMP[1] and cGMP.

## Materials and Methods

*Materials.* [8-$^3$H]cAMP (specific activity 16–23 Ci/mmol) and cyclic [8-$^3$H]cGMP (specific activity 4.1 Ci/mmol) were purchased from Schwarz/Mann (Orangeburg, N.Y.) and New England Nuclear (Boston, Mass.). [$^3$H]cAMP was purified by Dowex 1-X8 (Bio-Rad, 200–400 mesh) anion-exchange chromatography and stored at −20 °C in acid–50% ethanol. Snake (*Ophiophagus hannah*) venom, cAMP, and cGMP were obtained from Sigma (St. Louis, Mo.). 17$\beta$-Estradiol was purchased from Schwarz/Mann. DEAE-cellulose was purchased from Whatman and prepared as previously described

† From the Department of Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77025. *Received December 21, 1977.* This research was supported in part by National Institutes of Health Grants GM-21361 and HD-08615. Preliminary reports of portions of this work were presented at the 60th FASEB Meeting, Anaheim, April 1976 ((1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 610), and the 61st FASEB Meeting, Chicago, April 1977 ((1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 320).

‡ Recipient of a Faculty Development Award from the P. M. A. Foundation during a portion of this work.

§ Recipient of a National Institutes of Health Research Career Development Award (K04 HD-00099).

[1] Abbreviations used: cAMP, cyclic adenosine 3′:5′-monophosphate; cGMP, cyclic guanosine 3′:5′-monophosphate; Tris, tris(hydroxymethyl)aminomethane; Bes. *N,N*-bis(2-hydroxyethyl)2-aminoethanesulfonic acid; DEAE, diethylaminoethyl; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tricine, *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)-glycine.

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(Van Inwegen et al., 1976). Bio-Gel A-1.5m (100–200 mesh) was obtained from Bio-Rad (Richmond, Calif.). Ultrapure sucrose (density gradient grade) was obtained from Schwarz/Mann. All other reagents were of the highest grade commercially available.

*Preparation of Tissue.* Female Sprague Dawley rats (20–22 days of age) were obtained from Texas Inbred Mice Co., Houston, Texas. Rats were sacrificed by decapitation and the uteri were removed and stripped of adherent fat and mesentery and placed in 40 mM Tris–0.32 M sucrose (pH 8.0). Uteri were homogenized in the above buffer (1 uterus per mL) with a tight-fitted Duall type glass homogenizer and centrifuged at 105 000*g* for 1 h. Using these procedures uterine cytosol contains over 95% of the cAMP and cGMP phosphodiesterase activities present in the homogenate. Protein was measured by the method of Schacterle & Pollack (1973) using bovine serum albumin as a standard.

For in vivo studies saline (0.5 mL) or 2.5 $\mu$g of estradiol in 95% saline/5% ethanol (0.5 mL) was injected intraperitoneally. This dose of estradiol produces maximum uterotropic responses at all times after administration (Hisaw, 1959). Rats were sacrificed at the indicated times and cytosols prepared as described above. Rats used for longer term studies (72 h) were ovariectomized (at 22 days of age) 4 days before hormone administration in order to decrease variations in uterine weights, presumably occurring from secretion of small amounts of ovarian estrogens.

*Cyclic Nucleotide Phosphodiesterase Assay.* cAMP phosphodiesterase activity was measured by the radioisotope method of Thompson & Appleman (1971a). The assay mixture (0.4 mL) contained 5 mM $MgCl_2$, 40 mM Bes, pH 7.0, 5 mM $\beta$-mercaptoethanol, cAMP (containing approximately 150 000 cpm [$^3$H]cAMP per assay), and 0.1 mL of uterine cytosol (approximately 30–40 $\mu$g of protein). Reactions were initiated by the addition of enzyme; incubations were for 15 min at 30 °C and were terminated by boiling for 45 s. The [8-$^3$H]adenosine product was measured after treatment of the reaction mixture with snake venom (0.1 mg/mL) for 10 min and precipitation of unreacted substrate with anion-exchange resin as described previously (Thompson & Appleman, 1971a). All but kinetic studies used 0.25 $\mu$M cAMP substrate. The method of Weiss et al. (1972) was used where indicated to measure total cAMP phosphodiesterase activity with 200 $\mu$M cAMP as substrate. Reactions were linear with respect to time and protein content. As reported by Rutten et al. (1973) some recently purchased anion exchange batches may bind appreciable amounts of the adenosine or guanosine reaction product in a nonspecific manner. To minimize this problem a resin slurry containing 40% methanol was used as described by Thompson et al. (1976). The blank values obtained with this procedure do not exceed those in the absence of methanol and product recoveries were 90% or greater.

cGMP phosphodiesterase activity was measured by the radioisotope procedure as described above, except that incubations were carried out for 10 min. Unless otherwise indicated activity was measured with 1 $\mu$M cGMP (containing approximately 130 000 cpm [$^3$H]cGMP) as substrate.

*Sucrose Gradient Centrifugation.* Linear 5–20% sucrose gradients (5 mL) containing 50 mM Tris (pH 7.4) were prepared as described by Martin & Ames (1961). Cytosol was prepared in 40 mM Tris (pH 8.0) and 0.2 mL (approximately 0.5 mg of protein) was layered on each gradient. $^{14}$C-labeled ovalbumin (3.6 S) and $\gamma$-globulin (7.0 S) were used as sedimentation standards (Stancel & Gorski, 1974). Centrifugation was for 12–16 h at 40 000 rpm in a Beckman SW 50.1 rotor and 0.15-mL fractions were collected by puncturing the bottom of the tube.

*DEAE-Cellulose Chromatography.* DEAE-cellulose was equilibrated in a column (6 mm diameter, 2 mL bed volume) at pH 8.0 with 20 mM Tris-Cl–20 mM sodium acetate–10 mM sodium fluoride–0.1 mM dithiothreitol–5% glucose–30% ethylene glycol or at pH 6.0 with the same reagents in 20 mM Mes (Van Inwegen et al., 1976). Uterine cytosol (6 mg of protein) was diluted in either of the above buffers and applied, and the cellulose column washed with the equilibrating buffer until no protein was detectable in the effluent. After washing with a 50–200 mM sodium acetate gradient prepared in the equilibrating buffer enzyme activity was eluted with a 200–1000 mM sodium acetate gradient at a flow rate of 0.17 mL/min and 1.0-mL fractions were collected.

*Isoelectric Focusing.* Linear density gradients of either sucrose (10–40% w/v) or glycerol (25–70% v/v) containing 1.0 or 1.5% LKB carrier ampholytes (pH 3–10), respectively, were used in an LKB 8101 isoelectric focusing column. The anode solution was 0.5 N $H_2SO_4$ in 65% sucrose and the cathode solution was 1% (w/v) NaOH. Uterine cytosol (2.5 mL containing 3 mg of protein) was made 5% (w/v) in sucrose and applied to the column. A Brinkman Lauda K-2R circulating, cooling bath maintained the column at 2–4 °C. Constant voltage (580 V) was applied for 60 h using an LKB 3371E dc power supply. The amperage dropped tenfold during the first 24 h and thereafter remained constant. Fractions of 2.0 mL were collected and their pH and enzyme activity measured.

*Agarose Gel Filtration.* The resin was washed extensively with 5 mM Mes–100 mM sodium acetate–0.1 mM dithiothreitol–30% ethylene glycol adjusted to pH 6.4 until the pH and conductivity of the washings were identical with that of the buffer. Uterine cytosol (200 $\mu$L containing approximately 4 mg of protein) prepared in the above buffer was applied to the column (9 mm diameter, 20 mL bed volume). The column was eluted at 4 °C with the above buffer at a flow rate of 0.12 mL/min.

## Results

*General Properties of Uterine Cyclic Nucleotide Phosphodiesterase.* Figure 1 is a double-reciprocal plot (Lineweaver & Burk, 1934) obtained by assaying uterine phosphodiesterase at different substrate concentrations. By extrapolation of the data in both portions of the curve in the upper panel apparent $K_m$ values of 3 $\mu$M and 20 $\mu$M cAMP are obtained. Maximum velocities of 3 and 10 nmol min$^{-1}$ (mg of protein)$^{-1}$ were obtained for the low and high $K_m$ activities, respectively. In subsequent experiments, the cAMP phosphodiesterase activity observed at a substrate concentration of 0.25 $\mu$M was taken as a measure of the low $K_m$ activity.

In contrast to the cAMP phosphodiesterase activity, the uterine cGMP phosphodiesterase activity exhibits linear Lineweaver–Burk kinetics (Figure 1, bottom panel). The apparent $K_m$ for the cGMP phosphodiesterase is 3 $\mu$M and the maximum velocity is 5.6 nmol min$^{-1}$ (mg of protein)$^{-1}$.

Uterine cAMP and cGMP phosphodiesterase activities show very similar pH optima and divalent cationic requirements. The cAMP activity is maximum at pH 7.0 and the cGMP activity exhibits a similar maximum at pH 7.0–7.5 in several buffer systems (data not shown). Maximum activity of both cGMP and cAMP phosphodiesterase is observed at 1.25 mM $Mg^{2+}$ or 0.125 mM $Mn^{2+}$ indicating that $Mn^{2+}$ is a more effective divalent cation for these reactions. Either cation produces the same maximum activity for cAMP phosphodiesterase, while a greater maximum activity of cGMP phospho-

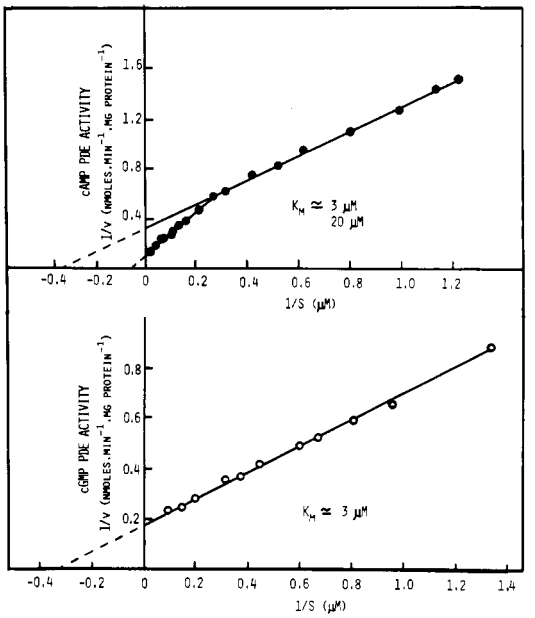


FIGURE 1: (Upper Panel) Representative Lineweaver-Burk plot of uterine cAMP phosphodiesterase (PDE) activity. Activity present in uterine cytosol was measured as described in Materials and Methods. $V_{max}$ values are 3 and 10 nmol min$^{-1}$ (mg of protein)$^{-1}$ for the low $K_m$ and high $K_m$ activities, respectively. (Lower Panel) Representative Lineweaver-Burk plot of uterine cGMP phosphodiesterase (PDE) activity measured as described in Materials and Methods. $V_{max}$ is 5.6 nmol min$^{-1}$ (mg of protein)$^{-1}$.

diesterase is observed in the presence of $Mg^{2+}$ (data not shown).

*Sedimentation Velocity.* A single symmetrical peak of phosphodiesterase activity with a sedimentation coefficient of 7.9 S is observed when uterine cytosol is centrifuged in a linear sucrose gradient (Figure 2). This peak contains both the cGMP and low $K_m$ cAMP phosphodiesterase (0.25 μM substrate) activities. This peak also contains the high $K_m$ cAMP phosphodiesterase (200 μM substrate) activity.

The profile illustrated in Figure 2 was obtained by centrifugation in a 5–20% sucrose gradient containing 0.05 M Tris, pH 7.4. Centrifugation was also performed at different pH (pH 6–8), ionic strengths (0.15–0.60 M KCl), protein concentrations (0.8–8.0 mg/mL) and in the presence of nucleotides or estradiol. The cGMP and cAMP phosphodiesterase activities comigrate and the sedimentation coefficient remains unchanged with each of these treatments.

*Gel Filtration.* When uterine cytosol is chromatographed on agarose A-1.5m, the profile of phosphodiesterase activity shown in Figure 3 is observed. A small amount of activity elutes with the void volume and probably represents aggregated material (Thompson & Appleman, 1971a,b). A single major peak which contains both cAMP and cGMP phosphodiesterase activity is eluted.

*DEAE-Cellulose Chromatography.* Sedimentation velocity and gel filtration revealed only a single form of uterine cyclic nucleotide phosphodiesterase (vide supra). Since both these techniques separate macromolecules on the basis of molecular weight and hydrodynamic properties, we further characterized uterine cyclic nucleotide phosphodiesterase by DEAE-cellulose chromatography and isoelectric focusing, which separate macromolecules on the basis of charge.

DEAE-cellulose chromatography performed at either pH 6 or pH 8 reveals a single major peak of phosphodiesterase activity (Figure 4) containing both the cAMP and cGMP hydrolytic activities.

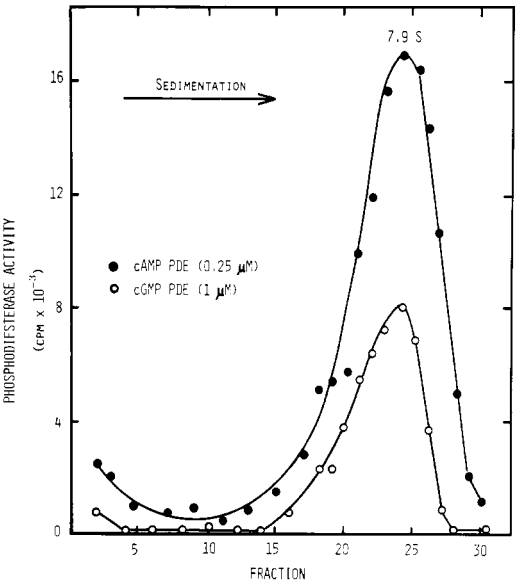


FIGURE 2: Sucrose gradient centrifugation of uterine cyclic nucleotide phosphodiesterase (PDE). Centrifugation and the assay of enzyme activities were performed as described in Materials and Methods. cAMP PDE activity was measured at 0.25 μM substrate and cGMP PDE activity at 1 μM substrate.

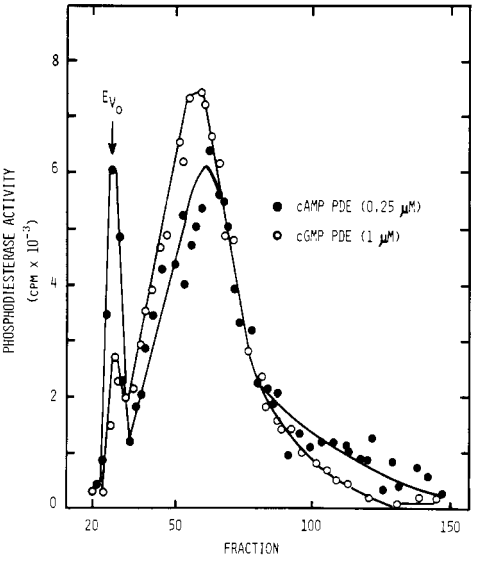


FIGURE 3: Agarose A-1.5m chromatography of uterine cyclic nucleotide phosphodiesterase (PDE). Chromatography and the assay of enzyme activities were performed as described in Materials and Methods. cAMP PDE activity was measured at 0.25 μM substrate and cGMP PDE activity at 1 μM substrate.

*Isoelectric Focusing.* Two peaks of phosphodiesterase activity with p*I* values of 5.2 and 5.8 are observed when uterine cytosol is fractionated by isoelectric focusing in 10–40% sucrose and 1% ampholytes (Figure 5). Both peaks contain cAMP and cGMP phosphodiesterase activity. Interpretation of these data is made difficult, however, since the pH 4 to pH 6 region of the gradient contains appreciable amounts of precipitate which may influence the profiles observed.

In an attempt to minimize this precipitation, isoelectric focusing was also performed in a gradient of 25–70% glycerol (Van Inwegen et al., 1976) containing 1.5% carrier ampholytes (data not shown). Using this procedure two peaks of phosphodiesterase activity with p*I* values of 5.1 and 6.5 are observed. Even with these conditions which alleviate precipitation in other systems (Van Inwegen et al., 1976), the activity in the

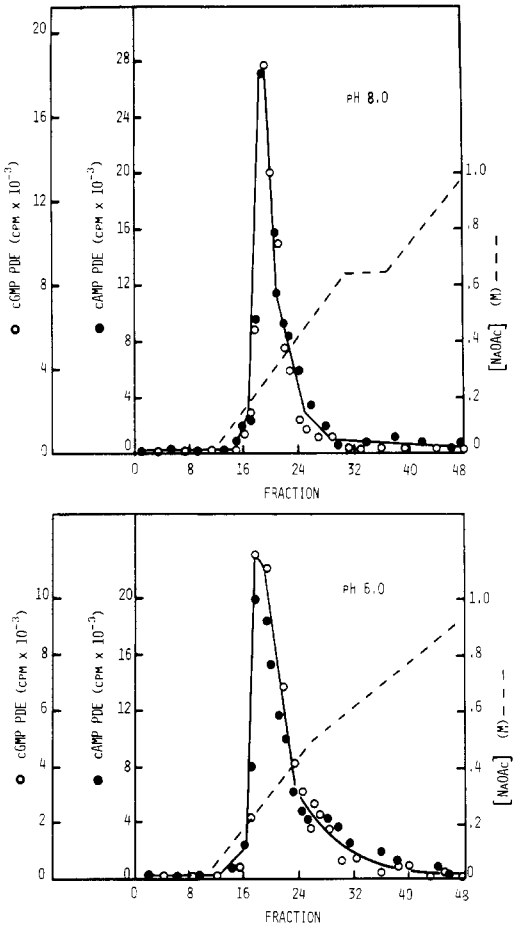


FIGURE 4: DEAE-cellulose chromatography of uterine cyclic nucleotide phosphodiesterase (PDE) at pH 8.0 (upper panel) and pH 6.0 (lower panel). Uterine cytosol was applied to the columns and eluted with sodium acetate (NaOAc) as described in Materials and Methods. cAMP PDE activity was measured at 0.25 $\mu$M substrate and cGMP PDE activity at 1 $\mu$M substrate.

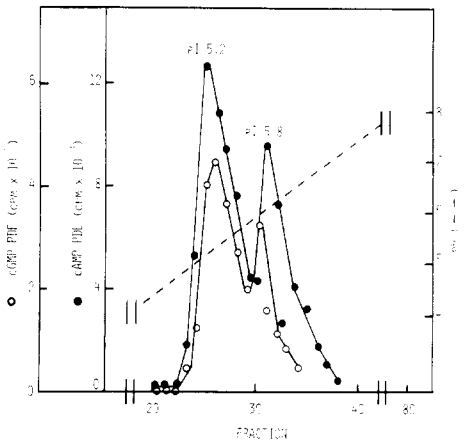


FIGURE 5: Isoelectric focusing of uterine cyclic nucleotide phosphodiesterase (PDE). Uterine cytosol was focused in a pH 3–10 gradient containing 10–40% sucrose and 1% ampholytes as described in Materials and Methods. cAMP PDE activity was measured at 0.25 $\mu$M substrate and cGMP PDE activity at 1 $\mu$M substrate.

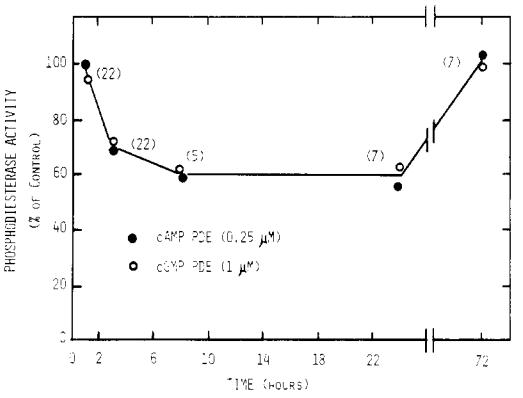


FIGURE 6: Effect of in vivo estradiol administration on uterine cyclic nucleotide phosphodiesterase (PDE) activity. Animals were treated with 2.5 $\mu$g of estradiol or saline at the indicated times prior to sacrifice. Uterine cytosol was prepared and assayed for cAMP PDE (0.25 $\mu$M substrate) and cGMP PDE (1 $\mu$M substrate) as described in Materials and Methods. Activity is expressed as the % of saline-treated controls at each time period. Values represent means and the $N$ values are given in parentheses; the SEM was less than 10% of the mean for each point. Control levels of activity were 186 pmol min$^{-1}$ (mg of protein)$^{-1}$ for cAMP PDE and 1800 pmol min$^{-1}$ (mg of protein)$^{-1}$ for cGMP PDE.

p$I$ = 6.5 peak appears to be associated with a precipitate, however, since 80–90% of the observed activity in this peak is removed by centrifugation of the collected fractions prior to assay. Both the p$I$ = 5.1 and p$I$ = 6.5 peaks contain cAMP and cGMP phosphodiesterase activity.

*Heat Denaturation Studies.* Incubation of uterine cytosol at 50 °C rapidly reduces the activity of cyclic nucleotide phosphodiesterase. Both the cAMP and cGMP phosphodiesterase activities are reduced at the same rate and to the same extent (data not shown).

*Effect of in Vivo Estradiol Administration on Uterine Phosphodiesterase.* The separation studies performed above indicate that the characteristics of uterine cAMP and cGMP phosphodiesterases are very similar if not identical. Since we had previously shown that estradiol administration in vivo leads to a decrease in uterine homogenate cAMP phosphodiesterase activity (Stancel et al., 1975), we further compared the effect of hormone treatment on both cAMP and cGMP phosphodiesterase activities in vivo over a 72-h period.

A rapid decrease in both the cAMP and cGMP phosphodiesterase activities is observed within 3 h after a single injection of 2.5 $\mu$g of estradiol. Both activities decline at the same rate (Figure 6) and remain depressed at the same level for 24 h before returning to control values at 72 h. While the mechanism by which estradiol decreases phosphodiesterase activity is not known, it is clear that both the cAMP and cGMP activities are affected in an identical fashion.

## Discussion

A key finding of our studies on uterine phosphodiesterase is the striking similarity between the cAMP and cGMP hydrolytic activities. A number of fractionation studies have demonstrated that the properties of the cAMP and cGMP phosphodiesterase are virtually identical. Furthermore, both phosphodiesterase activities are affected similarly under in vivo physiological conditions. These results strongly suggest that the hydrolysis of both cAMP and cGMP is catalyzed by a single enzyme entity. While a similar suggestion has been made for a form of phosphodiesterase activity present in rat lung (Fertel & Weiss, 1976), most mammalian tissues can be shown to contain distinct enzyme forms which preferentially catalyze the hydrolysis of either cAMP or cGMP (Thompson & Appleman, 1971a,b; Russell et al., 1973; Appleman et al., 1973; Appleman & Terasaki, 1975; Strada & Thompson, 1978; Wells & Hardman, 1977).

Uterine phosphodiesterase exhibits kinetic profiles similar to those reported for phosphodiesterase from other tissues. The soluble cAMP phosphodiesterase displays nonlinear Lineweaver-Burk plots with apparent $K_m$ values of 3 and 20 $\mu$M

(Figure 1). This is similar to values reported for hepatic phosphodiesterase (Thompson & Appleman, 1971b), but lower than the reported values for brain (Thompson & Appleman, 1971a; Weiss & Strada, 1972) and soluble rat kidney phosphodiesterases (Van Inwegen et al., 1976). In contrast cGMP phosphodiesterase exhibits linear kinetics with a $K_m$ of 3 $\mu$M (Figure 1). While these studies utilized 20–22 day old immature rats, the values may be dependent upon the age of the animal (Stancel et al., 1975) and the experimental procedures employed (Van Inwegen et al., 1976).

Uterine cAMP and cGMP phosphodiesterase activities both exhibit similar pH optima with a maximum at pH 7. This is lower than the optimum for other soluble forms of phosphodiesterase (Appleman et al., 1973), but similar to phosphodiesterases from renal brush border membranes (Filburn & Sacktor, 1976) and rat testes (Hintz & Strada, unpublished observations).

Both uterine phosphodiesterase activities require divalent cations for maximum activity as originally demonstrated for the enzyme from heart (Butcher & Sutherland, 1962) and brain (Cheung, 1967) and subsequently demonstrated in numerous other tissues. Either $Mg^{2+}$ or $Mn^{2+}$ fulfill this requirement for uterine phosphodiesterase. At low concentrations, $Mn^{2+}$ is a more effective stimulator than $Mg^{2+}$, but at saturating concentrations $Mg^{2+}$ produces identical (cAMP phosphodiesterase) or greater activity (cGMP phosphodiesterase) than $Mn^{2+}$. The concentrations of $Mg^{2+}$ and $Mn^{2+}$ which yield half-maximal activity are identical for both cAMP and cGMP phosphodiesterase.

Numerous attempts to separate these two activities on a structural basis have been unsuccessful (Figures 2–4) using either fresh or aged preparations; nor were these two activities differentiated by heat denaturation studies. We tentatively conclude, therefore, that uterine cytosol contains only a single form of phosphodiesterase which catalyzes the hydrolysis of both cAMP and cGMP. Preliminary studies have also shown that membrane associated uterine phosphodiesterase catalyzes the hydrolysis of both cAMP and cGMP and has catalytic properties similar to the soluble enzyme (unpublished observations).

Isoelectric focusing (Figure 5) revealed two peaks of phosphodiesterase activity. This could indicate that the uterus contains two forms of phosphodiesterase with very similar structures that are not resolved by other techniques, or it might be an artifact due to aggregation or the presence of precipitate in the pH gradient. Kidney phosphodiesterase, for example, can exhibit either one or two peaks of activity upon isoelectric focusing depending upon the experimental conditions (Van Inwegen et al., 1976). While further studies are required to resolve definitively these two possibilities it is clear that unlike other tissues (Pledger et al., 1974) both forms of uterine phosphodiesterase observed with isoelectric focusing catalyze the hydrolysis of cAMP and cGMP.

These results with the uterine enzyme system are at variance with data from numerous studies of phosphodiesterase from other sources. Sucrose gradient centrifugation (Van Inwegen et al., 1976; Thompson et al., 1976; Pichard & Cheung, 1976), DEAE-cellulose chromatography (Russell et al., 1973; Uzunov et al., 1973; Appleman et al., 1973; Russell & Pastan, 1974; Wells et al., 1975; Van Inwegen et al., 1976), gel filtration studies (Rosen, 1970; Thompson & Appleman, 1971a,b; Kakiuchi et al., 1971; Appleman et al., 1973; Van Inwegen et al., 1976), electrophoresis (Monn & Christiansen, 1971; Uzunov & Weiss, 1972; Goren & Rosen, 1972; Strada et al., 1974), and isoelectric focusing (Pledger et al., 1974; Van Inwegen et al., 1976) have all revealed multiple forms of phosphodiesterase. For most of these studies the separation techniques employed revealed at least one form of phosphodiesterase with a high affinity for cAMP and little or no capacity for the hydrolysis of cGMP, and a second form with a high affinity for cGMP and a much lower affinity for cAMP. Other studies revealed that aging causes changes in the relative distributions of different forms of phosphodiesterase (Russell et al., 1973; Thompson et al., 1976), and heat denaturation can distinguish different kinetic forms of phosphodiesterase (Bevers et al., 1974; Filburn & Sacktor, 1976).

It should be noted that other workers have published a preliminary report that the rat uterus contains two forms of phosphodiesterase which selectively hydrolyze cAMP and cGMP (Kraska et al., 1974). These divergent results could be due to differences in experimental procedures which are known to have major effects on the properties of phosphodiesterase (Van Inwegen et al., 1976) or to differences in age of the animals which are also known to affect the properties of uterine phosphodiesterase (Stancel et al., 1975). We employed immature rats while the previous study (Kraska et al., 1974) used mature animals. Uterine phosphodiesterase of monkey, however, appears to exist as a single enzyme form which catalyzes the hydrolysis of both cAMP and cGMP (Clarissa Beatty, personal communication).

In vivo studies also revealed that the activities of cAMP and cGMP phosphodiesterase are affected identically by estradiol (Figure 6). These results are also consistent with a single uterine enzyme catalyzing the hydrolysis of both cyclic nucleotides. It is not known if the decrease in phosphodiesterase activity following estradiol treatment is a key event which mediates subsequent uterine responses to the hormone, or whether the decrease in activity results secondarily from other effects of the hormone as previously suggested (Nichol & Goldberg, 1976). Studies in progress are aimed at answering this question and at elucidating the molecular basis for the observed changes in activity.

## References


Anderson, W. B., Russell, T. R., Carchman, R A., & Pastan, I. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 3802.

Appleman, M. M., & Terasaki, W. L. (1975) *Adv. Cyclic Nucleotide Res. 5,* 153.

Appleman, M. M., Thompson, W. J., & Russell, T. R. (1973) *Adv. Cyclic Nucleotide Res. 3,* 65.

Beavo, J. A., Hardman, J. G., & Sutherland, E. W. (1971) *J. Biol. Chem. 246,* 3841.

Bevers, M. M., Smits, R. A. E., Van Rign, J., & Van Wijk, R. (1974) *Biochim. Biophys. Acta 341,* 120.

Butcher, R. W., & Sutherland, E. W. (1962) *J. Biol. Chem. 237,* 1244.

Cheung, W. Y. (1967) *Biochemistry 6,* 1079.

Cheung, W. Y. (1971) *J. Biol. Chem. 246,* 2859.

Fertel, R., & Weiss, B. (1976) *Mol. Pharmacol. 12,* 678.

Filburn, C. R., & Sacktor, B. (1976) *Arch. Biochem. Biophys. 174,* 249.

Gardner, E. A., Thompson, W. J., & Stancel, G. M. (1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 610.

Goren, E. N., & Rosen, O. M. (1972) *Arch. Biochem. Biophys. 153,* 384.

Hisaw, F. (1959) *Endocrinology 64,* 276.

Kakiuchi, S., Yamazaki, R., & Yeshima, Y. (1971) *Biochem. Biophys. Res. Commun. 42,* 968.

Kakiuchi, S., Yamazaki, R., Yeshima, Y., & Uenishi, K. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 3526.

Kraska, R. C., Hyang-Yn, D., & Goldberg, N. (1974) *Pharmacologist 16,* 310.

Lineweaver, H., & Burk, D. (1934) *J. Am. Chem. Soc. 56,* 658.
Mangianello, V., & Vaughn, M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 269.
Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236,* 1372.
Monn, E., & Christiansen, R. O. (1971) *Science 173,* 540.
Nichol, S. E., & Goldberg, N. D. (1976) *Biochemistry 15,* 5490.
Pichard, A.-L., & Cheung, W. Y. (1976) *J. Biol. Chem. 251,* 5726.
Pledger, W. J., Stancel, G. M., Thompson, W. J., & Strada, S. J. (1974) *Biochim. Biophys. Acta 370,* 242.
Pledger, W. J., Thompson, W. J., & Strada, S. J. (1975a) *Nature (London) 256,* 729.
Pledger, W. J., Thompson, W. J., & Strada, S. J. (1975b) *J. Cyclic Nucleotide Res. 1,* 251.
Rosen, O. M. (1970) *Arch. Biochem. Biophys. 137,* 435.
Russell, T. R., & Pastan, I. (1974) *J. Biol. Chem. 249,* 7764.
Russell, T. R., Thompson, W. J., Schneider, F. W., & Appleman, M. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 1791.
Russell, T. R., Terasaki, W. L., & Appleman, M. M. (1973) *J. Biol. Chem. 248,* 1334.
Rutten, W. J., Schoot, B. M., & DePont, J. (1973) *Biochim. Biophys. Acta 315,* 378.
Schacterle, G. R., & Pollack, R. L. (1973) *Anal. Biochem. 51,* 654.
Stancel, G. M., & Gorski, J. (1974) *Methods Enzymol. 36,* 251.
Stancel, G. M., Thompson, W. J., & Strada, S. J. (1975) *Mol. Cell. Endocrinology 3,* 283.
Stevens, F. C., Walsh, M., Ho, H. C., Teo, T. S., & Wang, J. H. (1976) *J. Biol. Chem. 251,* 4495.
Strada, S. J., & Pledger, W. J. (1975) in *Cyclic Nucleotides in Disease* (Weiss, B., Ed.) p 3, University Park Press, Baltimore, Md.
Strada, S. J., & Robison, G. A. (1974) *Curr. Top. Exp. Endocrinol. 2,* 1.
Strada, S. J., & Thompson, W. J. (1978) *Adv. Cyclic Nucleotide Res. 9,* 265.
Strada, S. J., Uzunov, P., & Weiss, B. (1974) *J. Neurochem. 23,* 1097.
Thompson, W. J., & Appleman, M. M. (1971a) *Biochemistry 10,* 311.
Thompson, W. J., & Appleman, M. M. (1971b) *J. Biol. Chem. 246,* 3145.
Thompson, W. J., & Strada, S. J. (1977) in *Receptors and Hormone Action* (Birnbaumer, L., & O'Malley, B. W., Eds.) Vol. III, Academic Press, New York, N.Y. (in press).
Thompson, W. J., Little, S. A., & Williams, R. H. (1973) *Biochemistry 12,* 1889.
Thompson, W. J., Ross, C. P., Pledger, W. J., Strada, S. J., Banner, R. L., & Hersh, E. M. (1976) *J. Biol. Chem. 251,* 4922.
Uzunov, P., & Weiss, B. (1972) *Biochim. Biophys. Acta 284,* 220.
Uzunov, P., Shein, H. M., & Weiss, B. (1973) *Science 180,* 304.
Van Inwegen, R. G., Pledger, W. J., Strada, S. J., & Thompson, W. J. (1976) *Arch. Biochem. Biophys. 175,* 700.
Van Inwegen, R. G., Swafford, R. L., Strada, S. J., & Thompson, W. J. (1977) *Arch. Biochem. Biophys. 178,* 58.
Watterson, D. M., Harrelson, W. G., Keller, D. M., Sharief, F., & Vanamans, T. C. (1976) *J. Biol. Chem. 251,* 4501.
Weiss, B., & Strada, S. J. (1972) *Adv. Cyclic Nucleotide Res. 1,* 357.
Weiss, B., Lehne, R., & Strada, S. (1972) *Anal. Biochem. 45,* 222.
Wells, J. N., & Hardman, J. G. (1977) *Adv. Cyclic Nucleotide Res. 8,* 119.
Wells, J. N., Baird, C. E., & Hardman, J. G. (1975) *Biochim. Biophys. Acta 384* 430.