Regulation of cyclic nucleotide phosphodiesterase activity in myometrium from pregnant and spayed rhesus monkeys

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Summary. The activities of myometrial cyclic nucleotide phosphodiesterases (PDEs) and the sensitivity of these enzymes to the effector molecules, cGMP and cAMP, were determined in the 100 000 g supernatant of homogenates from pregnant and spayed rhesus monkeys. The specific activities (per mg nitrogen) of the myometrial cyclic nucleotide PDEs in the supernatant from spayed monkeys were higher than those from pregnant monkeys at all substrate levels studied. However, when calculated on the basis of the DNA content of the myometrium, which was 8 times higher in the spayed than in the pregnant animals, the specific activities were lower in the tissue from spayed animals. At substrate levels of 2.5 μM -cAMP, low levels of cGMP (0.1–1.0 μM) caused the same percentage increase in cGMP-PDE activity in both tissues. At high substrate levels of 100 μM -cAMP, 1 μM -cGMP inhibited only the cAMP-PDE from spayed monkeys, and the enzyme from spayed monkeys was more effectively inhibited by 10 and 40 μM -cGMP than was the enzyme from pregnant animals. The cGMP-PDE activity was inhibited by cAMP (1.0–50.0 μM), and the percentage inhibition with increasing levels of cAMP appeared to be similar in the two series. The levels of cGMP and cAMP that modify the rate of hydrolysis of the other nucleotide in rhesus myometrium seem to be within the physiological range for these compounds *in situ*. It therefore appears possible that cAMP and cGMP are each involved in regulating the degradation of the other nucleotide in rhesus myometrium.

Introduction

There has been considerable interest recently in the possibility that the contractile behaviour of some smooth muscles can be modulated by changes in the intracellular concentrations of the cyclic nucleotides (Hardman, 1977). Uterine smooth muscle is differentiated for a specific purpose and may or may not exhibit control mechanisms similar to those of other smooth muscles. Most tissues appear to contain at least two cyclic nucleotide phosphodiesterase (PDE) enzymes that vary in kinetic behaviour, in relative affinities for their substrates (cAMP and cGMP), in molecular weight, and in differential activation and inhibition (Van Inwegen & Thompson, 1975; Weiss & Hait, 1977; Goldberg & Haddox, 1977). Although the nucleotide cyclases, especially adenylate cyclase, are known to be under metabolic regulation, less is known about control of the PDEs. The evidence suggesting that cyclic nucleotide PDEs are important in the control of intracellular levels of cAMP and cGMP has been reviewed (Appleman & Terasaki, 1975; Weiss & Hait, 1977). Conversion of cAMP and cGMP to the 5'-nucleotide by

their PDEs is probably the major physiological mechanism known to terminate their action. We were therefore interested in studying possible regulatory mechanisms for PDE activity in primate myometrium since an understanding of nucleotide metabolism in this tissue might bring us closer to an understanding of the biochemistry of spontaneous and induced abortions in primates. The fact that the concentration of cyclic nucleotides and the enzymes that hydrolyse them are higher in rhesus myometrium than in adult rhesus skeletal muscle suggests that the cyclic nucleotides are particularly important in myometrial metabolism (Bocek, Young & Beatty, 1976; Beatty, Herrington & Bocek, 1977). The discovery that tissues of animals with certain diseases, e.g. aortae of hypertensive rats, adipocytes of congenitally obese mice, adipose tissue of diabetic mice and leukaemic lymphocytes, have abnormal PDE activities has focused attention on this enzyme (Weiss & Hait, 1977).

In this report we determined the total activity and the relative distribution of the cyclic nucleotide PDEs in subcellular fractions obtained by differential centrifugation of homogenates of myometrium from pregnant and spayed rhesus monkeys. We also studied the sensitivity of the myometrial cyclic nucleotide PDEs to the potential biological regulators, cGMP and cAMP, in myometrium from pregnant and spayed rhesus monkeys.

Materials and Methods

Uterine tissue was obtained at the time of Caesarean section from pregnant rhesus monkeys (*Macaca mulatta*) at 145 to 160 days gestation (average length of gestation is 167 days); the hormone levels, measured in the Oregon Regional Primate Research Center radioimmunoassay laboratory, were 576–960 pg oestradiol-17 β /ml and 1.7–4.3 ng progesterone/ml. The gestational age was calculated from the 2nd day of a 3-day mating period. The pregnant females were anaesthetized with halothane (2-bromo-2-chloro-1:1:1-trifluoroethane) (1% or less) in a mixture of 75% oxygen and 25% nitrous oxide. Biopsy specimens were obtained from the same area of the upper fundus within 5 min after opening the peritoneal cavity. The biopsy site was sometimes varied slightly to avoid the site of placental attachment. In the spayed monkeys, the uterus was removed 6 months or more after ovariectomy. The plasma concentrations of oestradiol-17 β and progesterone were <20 pg/ml and <0.2 ng/ml respectively (radioimmunoassay).

For measurement of cyclic nucleotide PDE activity and cyclic nucleotide levels *in situ*, several small samples of myometrium (about 200 mg) were excised, rinsed in normal saline (0.154 M-NaCl), and frozen in a Wollenberger clamp at -196°C . The shortest time taken to excise, rinse and freeze a small myometrial biopsy sample was 2–3 sec; it is not practical to freeze a large amount of myometrium this rapidly. When larger samples of myometrium were obtained from pregnant rhesus monkeys, they were cleaned and freeze-clamped at 3, 5 or 10 min. The uterus excised from the spayed monkeys was cut into quarters and the endometrium and myometrium were separated. Care was taken to prevent contamination of the two tissues with each other even if this meant sacrificing some of each tissue. The myometrium was sliced into small pieces and freeze-clamped. The muscle samples were then wrapped in aluminium foil and stored at -196°C until homogenization. Freezing did not change the cyclic nucleotide PDE activity in the whole homogenate or in the 650 and 100 000 g supernatant fractions, although Sakai, Makino & Tanaka (1977) reported a marked increase in cAMP-PDE of rat liver after freezing. At -196°C , the enzyme activity did not change over a 6-month period.

Preparation of cellular fractions. A 10% (w/v) homogenate in 0.25 M sucrose–10 mM-Tris (pH 7.4) was prepared. The frozen tissue was weighed in the frozen state, thawed in a small amount of cold buffer, and minced. One half of the required volume of medium was added, and the tissue was homogenized at 4°C in a Polytron ST 20 for 10 sec at 3/4 the maximum setting.

After centrifugation for 5 min at 500 *g* and 0°C, the supernatant was decanted; the precipitate was resuspended in the remainder of the sucrose-Tris buffer and homogenized again for 10 sec at the same setting. This second homogenate was centrifuged at 500 *g* for 5 min. The supernatants were combined and centrifuged for 10 min at 650 *g*. Aliquots of the whole homogenate and of the 650 *g* supernatant were saved for enzyme assay; the remainder was centrifuged at 10 000 *g* for 30 min and decanted. The supernatant was then centrifuged for 60 min at 100 000 *g* to obtain a supernatant and particulate fraction. The particulate fractions from the 10 000 *g* and 100 000 *g* centrifugations were washed by resuspension in 12 ml fresh buffer and recentrifugation. The resultant pellets were resuspended in 1–1.5 ml sucrose-Tris buffer. All fractions were quick frozen in dry ice-acetone and stored at -20°C; at this temperature the PDE activity remained unchanged for at least 3 months.

Cyclic nucleotide phosphodiesterase assays. Cyclic nucleotide PDE activities were determined by a modification of the two-stage assay of Loten & Sneyd (1970), as previously described (Beatty *et al.*, 1977). Total enzyme activities in the pregnant and spayed rhesus myometrium were compared in the fractions obtained by differential centrifugation since purification usually leads to a variable loss of activity. The tritiated cyclic nucleotides, hydrolysed by PDE to the [³H]5'-derivative, were converted to [³H]adenosine or [³H]guanosine and isolated with a Bio-Rad AG1-X2 (200–400 mesh) column for counting. With this system, recovery of cyclic nucleotides from the resin column averaged 103%. The amount of hydrolysed cyclic nucleotides was calculated from the specific activity of the substrate and expressed in terms of nmol cyclic nucleotide hydrolysed/min/mg nitrogen or protein. The concentrations of enzyme preparations were such that less than 35% of the substrate was hydrolysed. The analysis was run at pH 8.0. Substrate concentrations are indicated in the tables and figures; the level of [³H]cGMP was 0.07 µCi/assay tube (8.4 Ci/mmol); that of [³H]cAMP was 0.02 µCi/assay tube (22 Ci/mmol). Linearity was established for incubation time and homogenate dilutions. When the assay medium was made 1 mM with respect to Ca²⁺ there was no effect on cyclic nucleotide PDE activity; 3 mM Mg²⁺ was already present in the assay medium (Loten & Sneyd, 1970). Radioactivity in the blanks was usually less than 0.5% of the total added to the assay.

Total protein, nitrogen and deoxyribonucleic acid levels. Protein content of the various fractions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as a standard. The nitrogen content was determined on a Kjeldahl digest with a Technicon autoanalyser. When the protein content was calculated from the nitrogen value and compared with the protein value obtained by Lowry's technique, we found that Lowry's method gave significantly higher results, i.e. from 20 to 40%, depending on the subcellular fraction. Therefore, in reporting enzyme activities, we prefer to use nitrogen (Kjeldahl) as a reference base. The apparent $V_{\max}$, apparent K_m , and apparent K_i values were calculated by the method of least squares. A small sample (100–200 mg) of myometrium was homogenized in ice water with a Polytron homogenizer fitted with an ST-10 generator. An aliquot of the homogenate was analysed for DNA by the use of 3,5-diaminobenzoic acid dihydrochloride fluorescence (Cheek, 1975; Afting & Elce, 1978), and total nitrogen was determined, as already described, on another aliquot.

Cyclic nucleotide levels. Frozen tissues were homogenized in 6% trichloroacetic acid (TCA) with a Polytron homogenizer, fitted with a ST-10 generator (4°C). The homogenate was centrifuged, and the supernatant was extracted 4 times with 5 times its volume of water-saturated ether. The aqueous phase was evaporated to dryness at 70–75°C under a stream of nitrogen. The residue was diluted with 50 mM-acetate buffer (pH 6.2), and the cGMP concentration was determined by radioimmunoassay (New England Nuclear cGMP Kit). Cyclic AMP levels were assayed on the same TCA extract by a modification of the method of Gilman (1970), as previously described (Bocek *et al.*, 1976).

Statistical analysis. Differences between means were not considered significant unless the *P* value was <0.05.

Results

There was no difference in the activities of the cyclic nucleotide PDEs (per g wet wt) of the whole homogenate of pregnant rhesus myometrium and the 650 g supernatant. The distributions of the subcellular fractions of pregnant and spayed rhesus myometrium were similar, and over 79% of the activity was in the 100 000 g supernatant fraction (Table 1). Cyclic nucleotide PDE of rat myometrium has also been reported to be mainly in the cytosol fraction (Bär, 1974).

Table 1. Distribution of cyclic nucleotide phosphodiesterase activity (means of duplicate determinations, for 2 experiments) in the subcellular fractions of myometrial homogenates from pregnant and spayed rhesus monkeys

	0.75 μ M-cAMP		100 μ M-cAMP		20 μ M-cGMP	
	Pregnant	Spayed	Pregnant	Spayed	Pregnant	Spayed
Activity (nmol hydrolysed/g wet wt $\cdot$ min ⁻¹)						
Whole homogenate	10.2, 12.0		85, 97		43, 51	
650 g supernatant	10.7, 11.8	13.6, 14.8	83, 91	130, 142	47, 41	95, 106
Activity in subcellular fractions (%)						
100 000 g supernatant	91	89	79	87	98	90
10 000 g pellet	2.5		1.3		0.9	
100 000 g pellet	7.3	5.7	3.2	4.0	1.2	4.0

Samples were frozen 5–10 min after excision. The fractions were prepared by differential centrifugation as outlined in the 'Methods'.

Table 2. The activity of cAMP–phosphodiesterase and cGMP–phosphodiesterase in the 100 000 g supernatant fraction of homogenates of myometrium (frozen 5–10 min after excision) from pregnant and spayed rhesus monkeys

	cAMP–PDE activity (μ mol cAMP hydrolysed)		cGMP–PDE activity (μ mol cGMP hydrolysed)	
	/g N/min*	/g DNA/min†	/g N/min*	/g DNA/min†
	Substrate 2.5 μ M-cAMP		Substrate 0.8 μ M-cGMP	
Pregnant	2.0 $\pm$ 0.1	10.2	0.48 $\pm$ 0.07	1.9
Spayed	3.5 $\pm$ 0.3	3.0	2.10 $\pm$ 0.2	1.4
	Substrate 25 μ M-cAMP		Substrate 10 μ M-cGMP	
Pregnant	5.3 $\pm$ 0.4	27.0	3.3 $\pm$ 0.1	13.2
Spayed	11.0 $\pm$ 0.5	8.7	10.2 $\pm$ 0.9	6.6
	Substrate 100 μ M-cAMP		Substrate 20 μ M-cGMP	
Pregnant	7.8 $\pm$ 0.5	39.7	4.6 $\pm$ 0.8	18.3
Spayed	18.6 $\pm$ 0.5	15.0	14.5 $\pm$ 0.4	9.4

* Values are means $\pm$ s.e.m. for duplicate or triplicate assays on samples from 4 pregnant monkeys, and duplicate or triplicate assays on myometrium from 4 monkeys spayed for at least 6 months. Values for the spayed monkeys are significantly greater than those for the pregnant monkeys ($P < 0.01$).

† Calculated from the ratios of nitrogen in the 100 000 g supernate and the whole homogenate (0.23 and 0.28 for the pregnant and spayed tissue) and the values for DNA (1.34 $\pm$ 0.09 and 10.9 $\pm$ 1.8 mg/g wet wt for the pregnant and spayed tissue).

When calculated on the basis of the wet wt or the nitrogen content of the fraction analysed, the activity of the cAMP– and cGMP–PDE enzymes for the samples from the spayed animals was higher than that in the pregnant animals in the 650 g supernatant (Table 1) and the 100 000 g supernatant at all substrate levels tested (Tables 2 and 3). However, the concentration of DNA

was 8 times higher in myometrium from spayed than from pregnant rhesus monkeys (Table 4). Therefore, when the enzyme activities in the 100 000 g supernatant were calculated on the basis of the DNA content of the myometrium, the activity in samples from spayed animals was lower than that from pregnant animals (Table 2). The concentration of nitrogen and the percentage dry weight were also higher (about 20–30%) in spayed than in pregnant rhesus myometrium (Table 4).

Table 3. Kinetic values for cyclic nucleotide–phosphodiesterase enzymes of myometrium (frozen 5–10 min after excision) from 3 pregnant and 3 spayed rhesus monkeys

	cAMP-PDE*					
	Low affinity		High affinity		cGMP-PDE†	
	K_m (μ M)	V_{max} ‡	K_m (μ M)	V_{max} ‡	K_m (μ M)	V_{max} ‡
100 000 g supernatant						
Pregnant	18.7	10.9	4.6	5.8	7.6	9.3
Spayed	24.9	20.8	2.9	6.7	10.9, 1.7§ 5.4	21.8, 6.9§ 15.4
100 000 g pellet						
Pregnant	16.2	2.1	2.5	1.1	14.6	1.6
Spayed	25.8	4.5	1.6	1.3	5.2	1.6

* Substrate concentrations 0.8–100 μ M-cAMP.

† Substrate concentrations 0.6–20 μ M-cGMP.

‡ Velocities are in terms of nmol cyclic nucleotide hydrolysed/mg nitrogen min⁻¹.

§ Nonlinear plot.

Table 4. Mean $\pm$ s.e.m. concentrations of DNA and nitrogen and percentage dry weight of myometrium from pregnant (145–160 days) and spayed (6 months or more) rhesus monkeys (no. in parentheses)

	DNA (mg/g wet wt)	Nitrogen (g/100 g wet wt)	% Dry wt
Pregnant	1.34 $\pm$ 0.09 (14)	2.36 $\pm$ 0.10 (10)	17.1 $\pm$ 0.3 (10)
Spayed	10.9 $\pm$ 1.5 (5)	3.10 $\pm$ 0.2 (5)	22 $\pm$ 0.2 (5)
Significance	$P < 0.005$	$P < 0.005$	$P < 0.005$

Anomalous double-reciprocal plots, suggestive of multiple enzyme forms (Russell, Thompson, Schneider & Appleman, 1972), were observed for cAMP hydrolysis by the 100 000 g fractions of myometrium from pregnant and spayed monkeys (Table 3). The apparent K_m value was 4- to 16-fold greater for the high K_m than for the low K_m enzyme (100 000 g supernatant and particulate). The V_{max} values for the high K_m enzyme were only 2- to 3-fold greater than those for the low K_m enzyme. We found linear kinetics for cGMP-PDE activity of the myometrium of pregnant animals but this was not always true for that from spayed rhesus monkeys (Table 3). Two Lineweaver–Burk plots determined with the 100 000 g supernatant of myometrium from spayed monkeys were linear; however, a third was clearly non-linear and had two possible K_m and V_{max} values (Table 3). Beavo, Hardman & Sutherland (1970) also have data suggesting the presence of cGMP-PDE enzyme with a low K_m in the 1000 g particulate fraction of bovine heart, and other workers have reported curves with similar discontinuities with unpurified muscle enzyme preparations (Goldberg & Haddox, 1977).

The maximum rate of hydrolysis of cAMP and cGMP was similar in the supernatant fractions of myometrium from pregnant and spayed monkeys (Table 3). The $V_{\max}$ values for the cyclic nucleotide PDEs of the 100 000 g supernatant were at least 5-fold higher than the values for the 100 000 g particulate fractions.

For our study on the sensitivity of the cyclic nucleotide PDE enzymes to cAMP and cGMP we used the 100 000 g supernatant because most of the PDE enzyme activity in uterine smooth muscle appeared in this fraction (Table 1). Equal stimulation of cAMP-PDE activity with cGMP was observed at low substrate levels of cAMP in tissue from pregnant and spayed animals (Text-fig. 1a). The stimulatory effect of low levels of cGMP was also reflected in the $V_{\max}$ value for the high affinity cAMP-PDE of pregnant myometrium which increased from 5.8 to 8.7 nmol cAMP hydrolysed/mg nitrogen min^{-1} in the presence of 5 μM -cGMP. At substrate levels of 100 μM -cAMP, 1 μM -cGMP inhibited only the enzyme from spayed monkeys, and 10 and 40 μM -cGMP were more effective inhibitors of myometrial cAMP-PDE from spayed than from pregnant monkeys (Text-fig. 1b).

In contrast to the mixed effect of cGMP on cAMP-PDE activity, cAMP inhibited cGMP-PDE at both low and high substrate concentrations (Text-fig. 2). The percentage inhibition with increasing levels of cAMP appeared to be similar for both sample types. When double-reciprocal plots were studied they suggested that the inhibition was non-competitive for the enzyme from the pregnant animals and competitive for that from the spayed monkeys. Similar results were

Text-fig. 1. The effect of cGMP on the cAMP-phosphodiesterase activity is reversed at (a) low and (b) high substrate concentrations. The experiments were performed with the 100 000 g supernatant fraction of myometrial homogenates from pregnant (open columns) and spayed (stippled columns) rhesus monkeys. Vertical bars represent mean ± 1 s.e.m., 4 monkeys in each series, duplicate or triplicate assays. The asterisks indicate that addition of cGMP caused a significant change in enzyme activity ($P < 0.01$). The P values indicate a significant difference in the effect of cGMP in the tissue from the pregnant and the spayed animals.

Text-fig. 2. Inhibition of cGMP-phosphodiesterase activity increased with increasing levels of cAMP at (a) low and (b) high substrate levels. The experiments were performed with the 100 000 g supernatant fraction of myometrial homogenates of pregnant (open columns) and spayed (stippled columns) monkeys, 2 in each series, duplicate or triplicate determinations.

obtained with Dixon plots; the average K_i values were 40 μM and 31 μM respectively (data not shown).

The average ($\pm$ s.e.m.) cGMP level of quick-frozen (3 sec) pregnant rhesus myometrium was 56 ± 8 pmol/g wet wt or 2.9 ± 0.3 pmol/mg N ($n = 5$); the concentration was 40% lower when the samples were frozen 120 sec after excision. By 10 min after excision, the cGMP had decreased about 70%. The cAMP level of quick-frozen myometrium from pregnant monkeys was 504 ± 51 pmol/g wet wt, or 26 ± 2 pmol/mg N. Previously, we have reported that the average cAMP, 5–10 min after biopsy of pregnant rhesus myometrium, was 909 ± 95 pmol/g wet wt (Beatty, Bocek, Young & Novy, 1976). Cyclic nucleotide levels change so rapidly that interpretation of the results is difficult and one can only assume that a 3-sec sample reflects in-situ levels. Cyclic nucleotide concentrations in perfused rat hearts can be altered within 1 sec after hormone stimulation (Drummond, Duncan & Hertzman, 1966). We did not analyse myometrium from spayed rhesus monkeys for cyclic nucleotide levels.

Changes in enzyme activity were also observed with time. The activity (mean $\pm$ s.e.m.) of cAMP-PDE increased from 3 to 60 sec after biopsy by $36 \pm 2.8\%$, $39 \pm 4.4\%$, and $40 \pm 4.5\%$ at substrate levels of 0.75 and 100 μM-cAMP and 20 μM-cGMP respectively. However, in the next 10 min, i.e. during the period when our samples were obtained, there were only minimal changes in enzyme activity ($\pm 5\%$).

Discussion

Cyclic nucleotides have frequently been implicated in the control of smooth muscle contraction (Schultz & Hardman, 1976), and changes in the concentration of these compounds may be related to spontaneous and induced abortions. Goldberg *et al.* (1975) have suggested that cGMP promotes and cAMP inhibits smooth muscle contraction. Although this relationship is controversial (Diamond, 1977), Hardman (1977) believes that contraction of some smooth muscles is modulated by cyclic nucleotides. It has also been suggested that cAMP inhibits growth whereas cGMP has an opposite effect; however, the data are not conclusive (Pastan, Johnson & Anderson, 1975; Whitfield *et al.*, 1976). The metabolism of cyclic nucleotides in myometrium is of particular interest since myometrium is a unique tissue that demonstrates (1) a large and acute phase of contractile activity during birth and (2) rapid growth during pregnancy; for example the weight of the rhesus uterus increases from 3–4 g to a maximum of 100–130 g (Gillespie, Ramsey & Reynolds, 1949; Behrman, Seeds, Battaglia, Hellegers & Bruns, 1964). Marked changes in rat uterine weights occur during the oestrous cycle (Sim & Chantharaksri, 1973) and preliminary work in our laboratory suggests similar but smaller changes during the menstrual cycle of the monkey. Telefer & Hisaw (1957) studied rabbit endometrium and myometrium separately and reported an increase in the weights of both endometrium and myometrium when progesterone was administered to a spayed, oestradiol-treated animal.

Both the level of cAMP and cGMP and the activity of the cyclic nucleotide PDEs (in terms of wet wt or nitrogen) is much higher in pregnant rhesus myometrium than in adult rhesus skeletal muscle (Beatty *et al.*, 1977), a tissue that is stabilized in respect to growth. Evidence that high levels of cyclic nucleotides act to induce synthesis of their PDE enzymes may partly explain the high level of PDE activity in myometrium from pregnant and spayed rhesus monkeys (Pastan *et al.*, 1975; Schwartz & Passonneau, 1975). Since both the levels of cyclic nucleotides and the activities of the enzymes hydrolysing them are high, we must assume that the turnover is also high. This is surprising since a high level of metabolic activity is not usually associated with myometrium (Hamoir, 1977). However, if one of the functions of cGMP is to make energy available to the cell upon hydrolysis (Goldberg & Haddox, 1977), the high level of this compound in myometrium could be useful.

We have no explanation for the higher enzyme activity (in terms of wet wt or nitrogen) in spayed than in pregnant myometrium. However, in terms of DNA, the enzyme activity is lower in the spayed myometrium because the DNA concentration is so high. The problem of a base of reference has concerned experimenters for years and there is no simple answer. Different interpretations of the same data are possible when values are expressed in terms of different reference bases (Voorhees, Stawiski & Duell, 1973). To facilitate comparison of our data with that of others, we have reported the values for dry weight, nitrogen and DNA content of pregnant and spayed myometrium as well as the enzyme activities in terms of both nitrogen and DNA. Because we wished to compare total enzyme activity under different physiological conditions the subcellular fractions obtained by differential centrifugation were not further purified since this procedure usually leads to large and variable losses in activity (Singer, Dunn & Appleman, 1978). Furthermore, isolation procedures may change enzyme specificity (Goldberg & Haddox, 1977) and cause markedly different fractionation profiles (Van Inwegen, Pledger, Strada & Thompson, 1976). The kinetic values for the high- and low- K_m PDE in these semi-purified preparations were undoubtedly influenced by the presence of the other enzyme. When multiple forms of PDE are separated, significant amounts of cAMP are hydrolysed at low (0.1 – 1.0 μM) substrate concentrations by the "high- K_m " PDE (Wells & Hardman, 1977).

In agreement with studies on other tissues, cGMP and cAMP do modify the activity of the cyclic nucleotide PDEs in rhesus myometrium (Russell, Terasaki & Appleman, 1973; Sakai, Thompson, Lavis & Williams, 1974; Terasaki & Appleman, 1975). We were particularly interested to determine whether the concentrations that were active in myometrium were within a

physiological range. The intracellular level of cGMP in pregnant rhesus myometrium is about $0.1\ \mu\text{M}$, assuming uniform distribution in the intracellular water. Therefore, the concentrations of cGMP ($0.1\text{--}1.0\ \mu\text{M}$) in our experiments that stimulated cAMP-PDE are within the physiological range for myometrial cGMP. The myometrial cAMP-PDE enzyme in rhesus monkeys can be described in the same terms as those used by Appleman & Terasaki (1975) to describe the rat-heart enzyme, i.e. as a cGMP-activated cAMP phosphodiesterase. These workers suggested that this cGMP effect represents not only another example of the "Yin-Yang" phenomenon but also might actually be the mechanism for a number of reported antagonistic and contradictory actions of cAMP and cGMP. The inhibitory level of cGMP ($1\text{--}40\ \mu\text{M}$) appeared to be above the physiological range; however, compartmentalization may well occur. Inasmuch as cGMP can both stimulate and inhibit hydrolysis of cAMP, the cellular content of cAMP in both spayed and pregnant rhesus myometrium may be controlled by cGMP-induced changes in cAMP/PDE activity. The fact that $1\ \mu\text{M}$ cGMP stimulates cAMP-PDE as substrate levels of $2.5\ \mu\text{M}$ and inhibits at $100\ \mu\text{M}$ cAMP is evidence for the presence of two different cAMP-PDE enzymes.

Cyclic AMP in concentrations of $1\text{--}50\ \mu\text{M}$ inhibited hydrolysis of cGMP in the supernatant fractions of myometrium from both pregnant and spayed rhesus monkeys. Therefore, the level of endogenous cAMP ($1\ \mu\text{M}$) is high enough to inhibit hydrolysis of cGMP *in vivo*. If one of the mechanisms by which cGMP modulates cell function involves rapid changes in its cellular level, a means of regulating the removal of this nucleotide could be important (Goldberg & Haddox, 1977). Although the details are still unclear, it appears that both cAMP and cGMP are probably involved in regulating the degradation of the other nucleotide in rhesus myometrium, regardless of the level of the sex steroids, and that the myometrium of spayed rhesus monkeys is even more sensitive to the addition of cGMP than is that from pregnant monkeys.

There have been a number of clinical studies demonstrating the importance of the cyclic nucleotides in biological systems. Alterations in cyclic nucleotide levels have been related to calcium disorders, diabetes mellitus, diabetes insipidus, mental disorders, psoriasis, cancer and asthma (Voorhees, Duell, Stawiski & Harrell, 1974). Spontaneous abortion and dysmenorrhoea may eventually be added to this list. For example, if a high level of cAMP inhibits myometrial contraction and a high level of cGMP inhibits cAMP hydrolysis one can postulate that a high level of both cyclic nucleotides in myometrium would be beneficial in cases of spontaneous abortions and dysmenorrhoea. Weiss & Hait (1977) have suggested that determination of the biochemical properties of the cyclic nucleotide PDE isoenzymes in normal and diseased tissue may lead to development of a new class of selective therapeutic agents that act by altering the metabolism of cyclic nucleotides.

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