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RAT UTERINE CONTRACTILITY AND THE ACTIVITIES OF UTERINE ADENYL CYCLASE AND PHOSPHODIESTERASE DURING THE ESTRUS CYCLE*

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Abstract—A study has been made of the activities of rat uterus adenyl cyclase and phosphodiesterase, and the inhibitory effect of theophylline on uterine contractions at various stages of the estrus cycle. The activities of adenyl cyclase and phosphodiesterase at proestrus were found to be 2·52 ± 0·28 pmoles/min/mg of protein and 1·90 ± 0·30 nmoles/min/mg of protein, respectively. Activities of both the enzymes increased from proestrus to peak values at metestrus (early metestrus for adenyl cyclase and late metestrus for phosphodiesterase) and then fell until the following proestrus. Theophylline inhibition of oxytocin-induced maximum uterine contractions was found to be greatest at early metestrus. Similarly, the oxytocin concentration producing 40 per cent maximum contraction in the presence and absence of the theophylline

$$\left[\frac{\text{Oxytocin } (E_{40}) + \text{theophylline}}{\text{Oxytocin } (E_{40})}\right]$$

was also highest at early metestrus. These findings indicate that, at early metestrus, cellular turnover of cyclic AMP may be high and that the exaggerated inhibition of oxytocin-induced maximum contraction and the high value of

$$\left[\frac{\text{Oxytocin } (E_{40}) + \text{theophylline}}{\text{Oxytocin } (E_{40})}\right]$$

could be due to extensive accumulation of cyclic AMP produced from theophylline inhibition of phosphodiesterase.

EPINEPHRINE and caffeine have been shown to inhibit the contractions of estrogen-dominated rat uterus produced in response to oxytocin.[1,2] Epinephrine is known to stimulate adenyl cyclase,[3] while caffeine and theophylline are inhibitors of phosphodiesterase.[4] Both these two classes of drugs act to elevate the endogenous cyclic AMP which has been shown to be responsible for the inhibition of oxytocin-stimulated rat uterus.[1,2] Cyclic AMP itself has also been shown to inhibit the action of oxytocin on rat uterus.[5] Hence it seems that the level of adenyl cyclase and phosphodiesterase activities in the rat uterus could be of significance in determining the intrinsic tonus and responsiveness of the uterus to physiological and pharmacological stimuli. Accordingly, a study was designed to determine the level of adenyl cyclase and phosphodiesterase activities in the rat uterus at various stages of the estrus cycle. The inhibitory effect of theophylline on uterine contractions produced in response to oxytocin at the different stages of the estrus cycle was also investigated.

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MATERIALS AND METHODS

Chemicals. Adenosine 5′-triphosphate (ATP), adenosine 3′,5′-monophosphate (cyclic AMP), phosphoenol pyruvic acid and pyruvate kinase of specific activity equal to 425 Units/mg of protein were obtained from Sigma. [$^{14}C_8$]Adenosine 5′-triphosphate of specific activity equal to 50 mCi/m-mole, and [$^{14}C_8$]adenosine 3′,5′-monophosphate of specific activity equal to 49 mCi/m-mole were obtained from 1 CN. Adenylate deaminase was extracted and purified from rat muscle by the method of Smiley *et al.*[6] A specific activity of 320 Units/mg of protein of enzyme was obtained.

Enzymes extraction and assay. Three-month-old female Sprague–Dawley rats with a weight range of 160–200 g were used. The stages of their estrus cycle were determined by vagina smear. Rats were killed by cervical dislocation and their uteri were immediately removed, trimmed free of adhering fat and weighed to the nearest milligram. Each weighed uterus was minced into small pieces and homogenized in six times its weight of 20 mM Tris–HCl buffer, pH 7·4, containing 10 mM KCl, 4 mM $MgCl_2$ and 10 mM theophylline at maximum speed for 3 min using a 5-ml microchamber of a Sorval micro-homogenizer attachment. The homogenate was dialyzed for 2 hr in 1 l. of the same buffer.

Adenyl cyclase. Adenyl cyclase activity was determined by the method described by Dousa and Rychlik.[7] Dialyzed homogenate (0·2 ml), containing 2–3 mg of protein, was added to 0·1 ml of substrate mixture to give 0·3 ml of final assay mixture containing 50 mM Tris–HCl, 7·4, 2 mM [$^{14}C_8$]ATP (sp. act. equal to 1·0 mCi/m-mole), 4 mM $MgCl_2$, 10 mM theophylline, 10 mM NaF, 10 mM phosphoenol pyruvic acid and 10 units of pyruvate kinase. Assays were done in triplicate and at 30°. After 30 min the enzyme reaction was stopped by immersing the tubes in a paraffin bath at 100° for 3 min. Fifty μg of carrier cyclic AMP was added to each tube and precipitated proteins were separated by centrifugation. The supernatant was spotted onto Whatman 3 MM paper (23 × 57 cm) with a distance of 7 cm between each spot. The remaining sediment was washed twice with 0·15 ml of distilled water plus carrier. The supernatant of the washings was transferred to the same starting point. The chromatograms were developed descendingly in isopropanol–NH_3–H_2O (7:2:1), isopropanol–HCl–H_2O (65:16·7:18·3), and butanol–water (86:14), respectively, as described by Dousa and Rychlik.[7] The separated cyclic AMP spots were detected under ultra-violet light and each spot was cut into small pieces directly into vial. Radioactivity was measured with a Packard liquid scintillation counter using 10 ml of Bray's solution as scintillant.

An experiment on adenyl cyclase activity vs incubation time, using 0·2 ml of homogenate, was initially carried out. From this experiment the incubation time of 30 min was chosen for subsequent assays of the enzyme as the formation of cyclic AMP under the assay conditions was linearly related to time up to 40 min. The recovery of cyclic AMP of the entire assay procedure was also determined. A known amount of [$^{14}C_8$] cyclic AMP was added to five tubes of boiled homogenate and the process of separating cyclic AMP from the mixture was carried out as described above. A recovery of 68 per cent was obtained.

Phosphodiesterase. The homogenate used for phosphodiesterase assay was prepared as described above except that the Tris buffer was without theophylline. Connective tissues in the dialyzed homogenate was removed by centrifugation at 90 *g* for 20 min. Phosphodiesterase activity was assayed spectrophotometrically as described by Drummond and Perrott-Yee.[8] Homogenate (0·1 ml) was added to 1·4 ml of substrate mix-

ture incubated at 30° in a 0·5 cm cuvette. The final volume of 1·5 ml of assay mixture contained 20 mM Tris–HCl, pH 7·4, 2 mM $MgCl_2$, 2 mM KCl and 10 units of adenylate deaminase. Extinction changes at 265 nm were recorded with a Beckman DK 2A spectrophotometer. The molar extinction change when AMP was converted to IMP in 20 mM Tris–HCl, pH 7·4, as determined experimentally was $7{\cdot}06 \times 10^3$ cm^{-1} m^{-1}. The adenylate deaminase used was in excess as further increase in amount of the coupled enzyme did not increase the rate of the overall reaction.

Measurement of uterine contractions. Uteri of rats from various stages of the estrus cycle were set up for isometric contraction as described by DeJalon *et al.*[9] A load of 1 g was used and the uterus was left to equilibrate for 15 min in Tyrode's solution before oxytocin of various doses (0·04–20·48 mU/ml) was used to produce a dose–response curve. Contraction of the myometrium produced by each dose was allowed for 3–5 min. After each dose of oxytocin, the uterus was washed three times with Tyrode's solution. The experiment was repeated using 50 mg/100 ml (w/v) of theophyllinized Tyrode for bath solution.

Protein. Protein in the uterine extracts was determined by the method of Lowry *et al.*[10] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Figures 1 and 2 depict adenyl cyclase and phosphodiesterase activities during the estrus cycle. Specific activity as well as activity per g of uterus of adenyl cyclase increased from proestrus to peak value at early metestrus, and then fell until the following proestrus. Phosphodiesterase activity varied in a similar way except that peak value was at late metestrus. The variation of the two enzymes during the estrus cycle was not the result of the variation of protein or of uterus weight. Average uterine weight

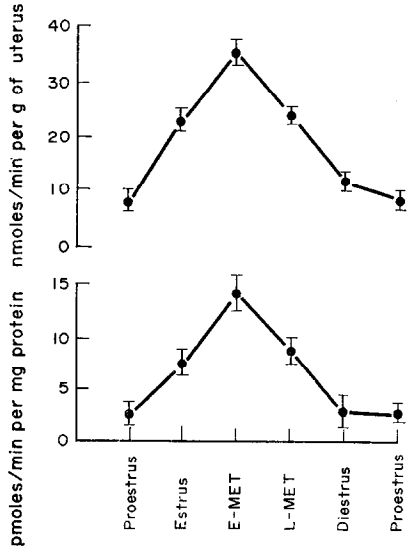

FIG. 1. Activity of rat uterine adenyl cyclase at various stages of the estrus cycle. E-Met and L-Met represent early metestrus and late metestrus respectively. Each point is an average of four to five separate determinations. The vertical bars represent standard deviations.

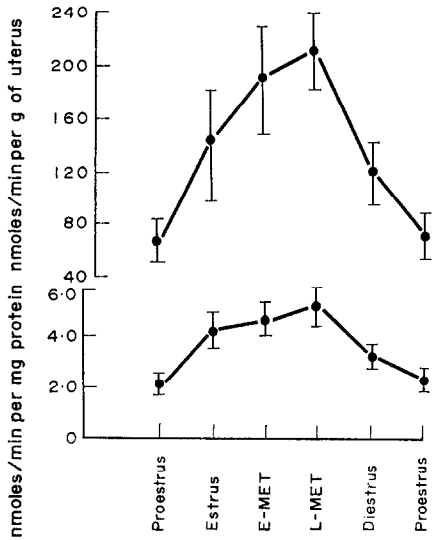

FIG. 2. Activity of rat uterine phosphodiesterase at various stages of the estrus cycle. E-Met and L-Met represent early metestrus and late metestrus, respectively. Each point is an average of four to five separate determinations. The vertical bars represent standard deviations.

at proestrus was 0·46 ± 0·09 g which is, respectively, 1·5- and 2-times higher than those at early metestrus and late metestrus (Table 1). Hence even if the activities of the two enzymes were to remain constant, the decrease in uterine weight from proestrus to metestrus could not have caused a 6-fold increase in adenyl cyclase and a 3·5-fold increase in phosphodiesterase activities. Similarly, the small variation of protein concentration of the uterine extracts could not have caused the observed variation of the two enzymes (Table 1).

Theophylline at 50 mg/100 ml (w/v) was found to inhibit uterine contractions produced in response to oxytocin. All the dose–response curves of oxytocin in the presence of theophylline of each estrus stage were shifted to the right and none reached the maximum response of the uninhibited curves (Fig. 3). Inhibition of maximum response

TABLE 1. WEIGHT OF UTERUS AND PROTEIN CONCENTRATION OF UTERINE EXTRACTS AT VARIOUS STAGES OF THE ESTRUS CYCLE

	Protein		Uterine wt
	(mg/ml)*	(mg/ml)†	(g)
Proestrus	6·9 ± 0·6	22·2 ± 2·6	0·46 ± 0·09
Estrus	7·8 ± 0·8	16·7 ± 0·6	0·43 ± 0·13
Early metestrus	8·7 ± 0·9	17·0 ± 1·1	0·32 ± 0·05
Late metestrus	8·8 ± 0·8	16·7 ± 0·8	0·23 ± 0·12
Diestrus	9·0 ± 1·1	15·3 ± 2·4	0·29 ± 0·11

* Ninety *g* homogenate.
† Unspun homogenate.

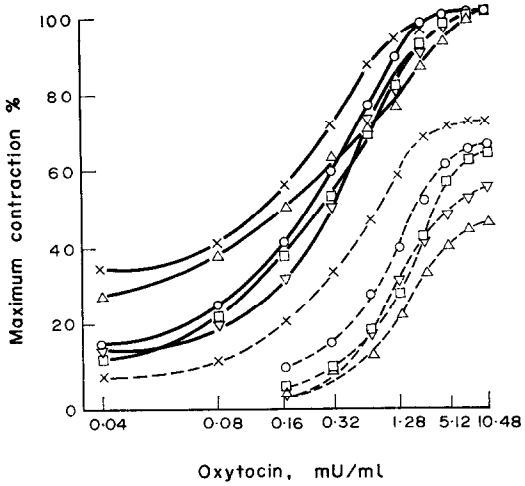

FIG. 3. Dose–response curves of uterine contraction to oxytocin in the absence (solid lines) and presence of 50 mg/100 ml of theophylline (broken lines) of various estrus stages (○——○, proestrus; □——□, estrus; △——△, early metestrus; ▽——▽, late metestrus; ×——×, diestrus). Each graph is an average plot of five to six separate experiments.

by theophylline when measured was found to increase from proestrus to maximum value at early metestrus and then decrease to the lowest value at diestrus. Similarly, the concentration of oxytocin producing 40 per cent maximum contraction in the presence and absence of 50 mg/100 ml (w/v) of theophylline

$$\left[\frac{\text{Oxytocin } (E_{40}) + \text{theophylline}}{\text{Oxytocin } (E_{40})}\right]$$

was also found to be highest at early metestrus and lowest at diestrus.

Since cyclic AMP has been shown to antagonize the action of oxytocin on rat uterus[5] and since theophylline is an inhibitor of phosphodiesterase,[4] we suggest that the observed inhibition of oxytocin-induced uterine contractions could possibly be caused by accumulated endogenous cyclic AMP. The results also indicate that this inhibition is extensive at periods when the activities of adenyl cyclase and phosphodiesterase are high. For example, the inhibition is greatest at early metestrus which corresponds to the period of peak and near-peak activities of adenyl cyclase and phosphodiesterase, respectively (Figs. 1 and 2). The high activities of the two enzymes at early metestrus could denote that the turnover of cellular cyclic AMP is both rapid and high. Hence inhibition of phosphodiesterase at early metestrus would result in extensive accumulation of cyclic AMP which could cause the observed pronounced inhibition of oxytocin-induced uterine contractions.

It is interesting to note that similar but not identical experiments carried out by Coutinho and Lopes[11] on human uterus showed that the human uterus displayed greater sensitivity to the inhibitory effect of aminophylline during the late proliferative and early luteal phases than the other phases of the menstrual cycle. The late proliferative and early luteal phases of the menstrual cycle are the ovulatory and post-ovulatory periods which correspond to the early and late metestrus of the rat estrus cycle.

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