CHANGES IN CYCLIC ADENOSINE MONOPHOSPHATE—PHOSPHODIESTERASE ACTIVITY IN NONPREGNANT AND PREGNANT HUMAN MYOMETRIUM

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Reprinted from
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY,
St. Louis

Vol. 157, No. 3, pp. 733-738, Sept., 1987

Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium

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Cyclic adenosine monophosphate–phosphodiesterase is the enzyme responsible for cyclic adenosine monophosphate degradation. We investigated the kinetic behavior of this enzyme in the myometrium of women who were nonpregnant, pregnant at term not in labor, and pregnant at term in active labor. Phosphodiesterase activity was measured in the 100,000 g supernatant by the two-step isotopic procedure. The Km (Michaelis constant) value remains essentially unchanged from the nonpregnant to the pregnant state and subsequent labor in both the low and the high affinity enzymes. During pregnancy the V max (maximum velocity) is 75% less than in the nonpregnant state ($p < 0.005$) and remains unchanged during labor. This is true for both the high and the low affinity enzymes. These changes in the kinetic characteristics of the cyclic adenosine monophosphate–phosphodiesterase are indicative of noncompetitive inhibition. We conclude that this inhibition may be interpreted as part of the mechanism for uterine smooth muscle relaxation and pregnancy maintenance. (AM J OBSTET GYNECOL 1987;157:733-8.)

Key words: Human myometrium, phosphodiesterase activity, pregnancy

Pregnancy transforms the human uterus from a small, almost solid organ in the nonpregnant state to a large saccular organ with a volume of approximately 5 liters.[1] This dramatic change is characterized by relaxation along with hypertrophy and hyperplasia of the smooth muscle fibers of the uterine wall. Although the contribution of cyclic nucleotides to uterine growth in pregnancy has not been completely determined, increasing evidence suggests that cyclic adenosine monophosphate may play an important role in myometrial relaxation through the effects of this compound on intracellular ionized calcium concentration and myosin light-chain kinase activity.[2, 3] These actions include the following concepts: Cyclic adenosine monophosphate is thought to be the second messenger for β-adrenergic agonists that stimulate adenylate cyclase and induce relaxation. Increasing levels of cyclic adenosine monophosphate promote ionized calcium uptake by the sarcoplasmic reticulum, producing a decrease in cytosolic ionized calcium and thus promote relaxation. Finally, cyclic adenosine monophosphate–mediated phosphorylation of myosin light-chain kinase inhibits this enzyme's activity and promotes smooth muscle relaxation.[4]

Adenylate cyclase catalyzes production of cyclic adenosine monophosphate and phosphodiesterase catalyzes cAMP degradation. For a given production of cyclic adenosine monophosphate, the intracellular levels will depend on the activity of phosphodiesterase. Although phosphodiesterase activity in human myometrium at term has been measured,[5, 6] there have been no studies comparing phosphodiesterase activity from pregnant and nonpregnant human myometrium. This study was undertaken to investigate this matter and to compare the enzymatic activity between samples taken from women in active labor and samples from patients not in labor.

Material and methods

Subjects. Myometrium was obtained from the anterior wall of the uterine corpus of six nonpregnant women who underwent hysterectomy for benign gynecologic indications (adenomatous hyperplasia [two], uterine prolapse [one], endometriosis [two], and adenomyosis [one]). The specimen was obtained immediately after the uterine vessels were ligated. Myometrium was also obtained from uteri of six women not in labor who underwent elective repeat lower segment transverse cesarean section at term and from six women in labor who underwent primary lower segment transverse cesarean section for various obstetric indications (high breech in labor, active labor with maternal herpetic genital lesions, cephalopelvic disproportion, and fetal distress). All nonpregnant patients had an unremarkable medical history and did not receive any medications before hysterectomy. All six patients were operated on while under general anesthesia. All pregnant patients were healthy and their pregnancies were un-

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Presented at the Seventh Annual Meeting of The Society of Perinatal Obstetricians, Lake Buena Vista, Florida, February 5-7, 1987.
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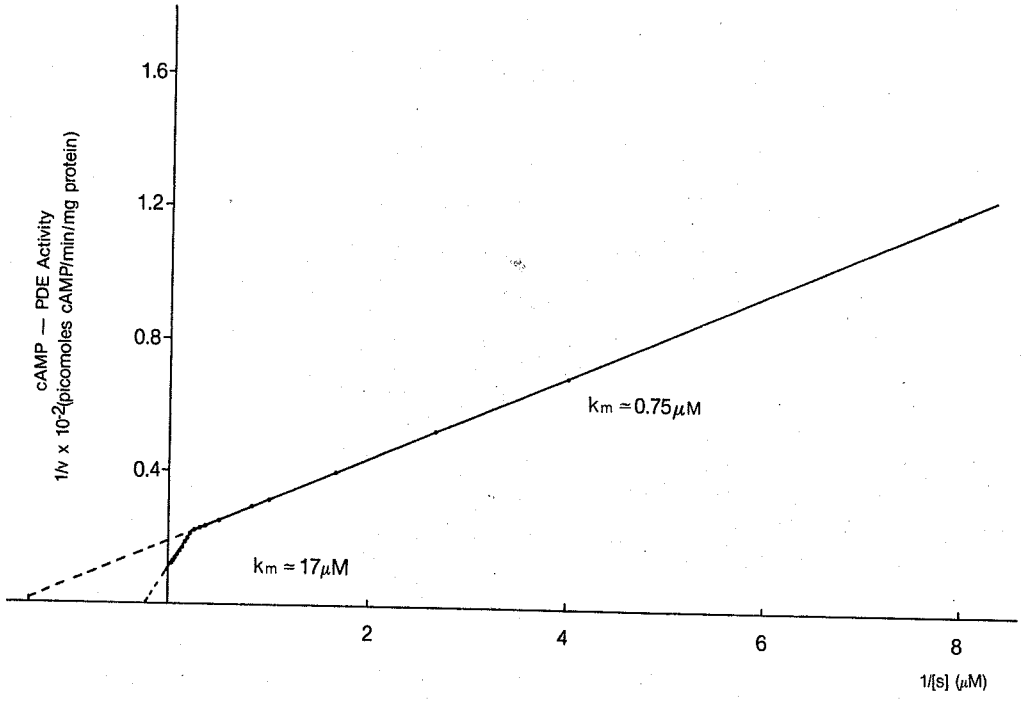

Fig. 1. Representative Lineweaver-Burk plot of human uterine cyclic adenosine monophosphate–phosphodiesterase activity versus substrate concentration. Evidence of two enzymatic forms with apparent Km values of approximately 0.75 and 17 μmol/L correspond to the high and low affinity enzyme, respectively (nonpregnant myometrium).

complicated until the time of delivery. None of the patients received uterotonic agents or other drugs. The procedure was performed with the patients under epidural anesthesia. The study was approved by the Clinical Research Practices Committee and each patient gave informed consent.

Tissue. The tissue biopsy specimen was taken from the upper edge of the transverse uterine incision from pregnant patients and from the lower part of the anterior uterine wall from nonpregnant patients. The tissue was rinsed in saline solution, blotted, and immediately frozen in a dry ice–acetone bath (−65° C). The entire procedure was performed within 60 seconds. Subsequently the tissue was stored at −70° C until assayed.

A 10% (wt/vol) homogenate in 0.25 mol/L sucrose, −10 mmol/L Tris hydrochloride (pH 8) was prepared (homogenization buffer). The frozen tissue was weighed in the frozen state, thawed in a small amount of buffer, and minced. The tissue was then homogenized at 4° C in a Polytron homogenizer for 30 seconds at three fourths of the maximum setting. The homogenate was then centrifuged for 60 minutes at 100,000 g and 4° C. Part of the supernatant was immediately assayed and the rest was stored at −70° C as a 1:1 solution in Tris hydrochloride–bovine serum albumin (0.5 mg bovine serum albumin per milliliter of 40 mmol/L Tris hydrochloride, pH 8). It was determined that freezing of the tissue and the homogenate did not change the enzyme activity for up to 6 months. The amount of protein was measured by a rapid and sensitive method that used the principle of protein-dye binding.[7]

Phosphodiesterase activity. Phosphodiesterase activity was measured in the supernatant by the two-step isotopic procedure as described previously by Thompson et al.[8] All assays, conducted in duplicate, were carried out in conditions of linearity with respect to time and protein concentration, allowing measurements of the initial rate of the reaction.

The assay mixture (0.4 ml) contained 5 mmol/L $MgCl_2$, 40 mmol/L Tris hydrochloride (pH 8), 4 mmol/L 2-mercaptoethanol, unlabeled cyclic adenosine monophosphate in different concentrations, labeled tritiated cyclic adenosine monophosphate (containing at least 100,000 cpm), and 0.1 ml of uterine cytosol. Reactions were initiated by the addition of enzyme; incubations were for 10 minutes at 30° C and were terminated by boiling for 45 seconds. After the addition of 0.1 ml of snake venom nucleotidase (1 mg/ml), the mixture was incubated for 10 minutes at 30° C and then placed in an ice bath. The entire content of the tubes was then transferred to Dowex 1-X8 (200 to 400 mesh) columns prepared in Pasteur pipettes (1 ml of 1:4 resin slurry was used to make each

column). The tritiated adenosine was then eluted with 2 ml of high-performance liquid chromatography grade methanol. The radioactivity of the eluate was determined in a liquid scintillation counter. In the range of substrate concentration from 0.125×10^{-6} to 100×10^{-6} mol/L, apparent Km (Michaelis constant) and maximum velocity values were calculated by Lineweaver-Burk plots by means of the computer program ENZPACK (Elsevier-BIOSOFT).

Statistical analysis. The differences in Km and maximum velocity values among the different groups were analyzed by one-way analysis of variance and Tukey's least significant difference test. A $\log_{10}$ transformation of the data was carried out to satisfy the prerequisite of homogeneity of variance when analysis of variance was used. A p value of ≤ 0.05 was considered significant.

Results

Kinetic properties of uterine cyclic adenosine monophosphate–phosphodiesterase. Kinetic analysis of cyclic adenosine monophosphate–phosphodiesterase activity versus cyclic adenosine monophosphate concentration by double reciprocal plots according to Lineweaver-Burk revealed nonlinear kinetic behavior suggestive of either multiple enzymatic forms or one form with negative cooperativity (Fig. 1). In nonpregnant myometrium in the range of cyclic adenosine monophosphate concentration from 0.125 to 4 μmol/L, an apparent Km value of 0.752 ± 0.046 μmol/L and maximum velocity of 2328 ± 197 pmol of cyclic adenosine monophosphate/min/mg of protein were obtained corresponding to the high affinity form of enzymatic activity. In the range from 5 to 100 μmol/L an apparent Km value of 17.68 ± 1.57 μmol/L and maximum velocity of 13,255 ± 1006 pmol/min/mg of protein were obtained corresponding to the low affinity form of enzymatic activity (Figs. 2 and 3).

In pregnant myometrium at term but not in labor the corresponding high affinity values were Km of 0.758 ± 0.038 μmol/L and maximum velocity of 604 ± 86 pmol/min/mg of protein. The corresponding low affinity values were Km of 22.31 ± 6.24 μmol/L and maximum velocity of 3570 ± 876 pmol/min/mg of protein (Figs. 2 and 3).

Similarly in pregnant myometrium in labor the high affinity values were Km of 0.674 ± 0.042 μmol/L and maximum velocity of 502 ± 100 pmol/min/mg of protein and the low affinity values, Km of 23.33 ± 4.44 μmol/L and maximum velocity of 2937 ± 464 pmol/min/mg of protein. The differences among the Km values were not significant but the differences in maximum velocity between nonpregnant and both pregnant groups were significant (analysis of variance, $p < 0.005$) (Figs. 2 and 3). These changes are suggestive of noncompetitive inhibition of uterine cyclic adenosine monophosphate–phosphodiesterase during pregnancy (Fig. 4).

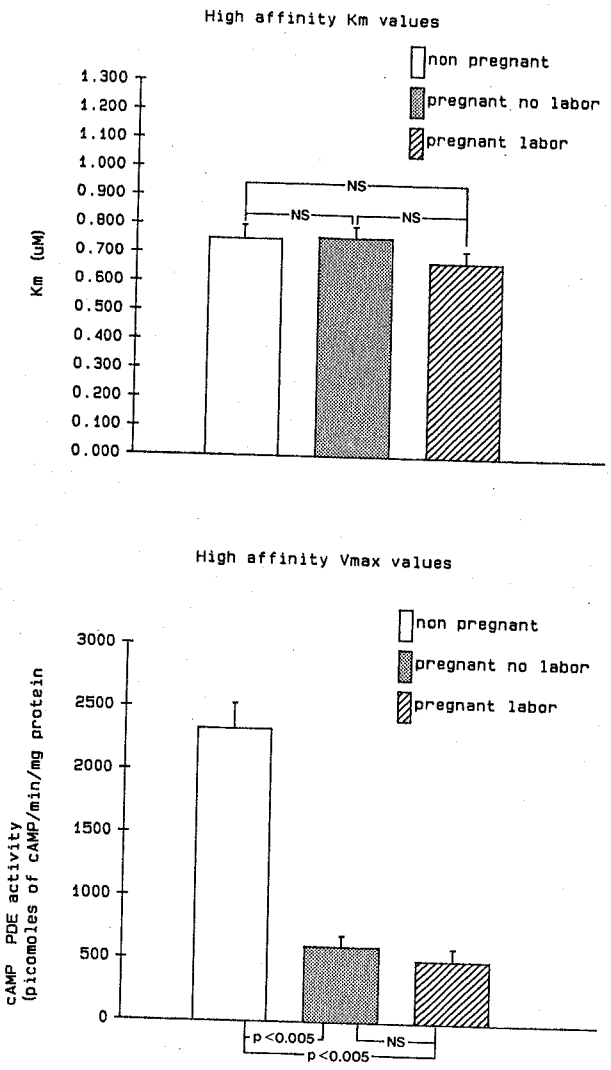

Fig. 2. *Top,* High affinity Km is similar in all three conditions. *Bottom,* High affinity, maximum velocity during pregnancy is approximately 75% less than in the nonpregnant state.

Comment

Normal pregnancy is a unique physiologic phenomenon that provokes major functional and anatomic changes in several organ systems. These changes create an environment necessary for the normal development of the conceptus and its maintenance in utero until maturity is accomplished. Cyclic nucleotides in general and especially cyclic adenosine monophosphate are generally thought to be involved in the control of the important structural and functional changes that occur

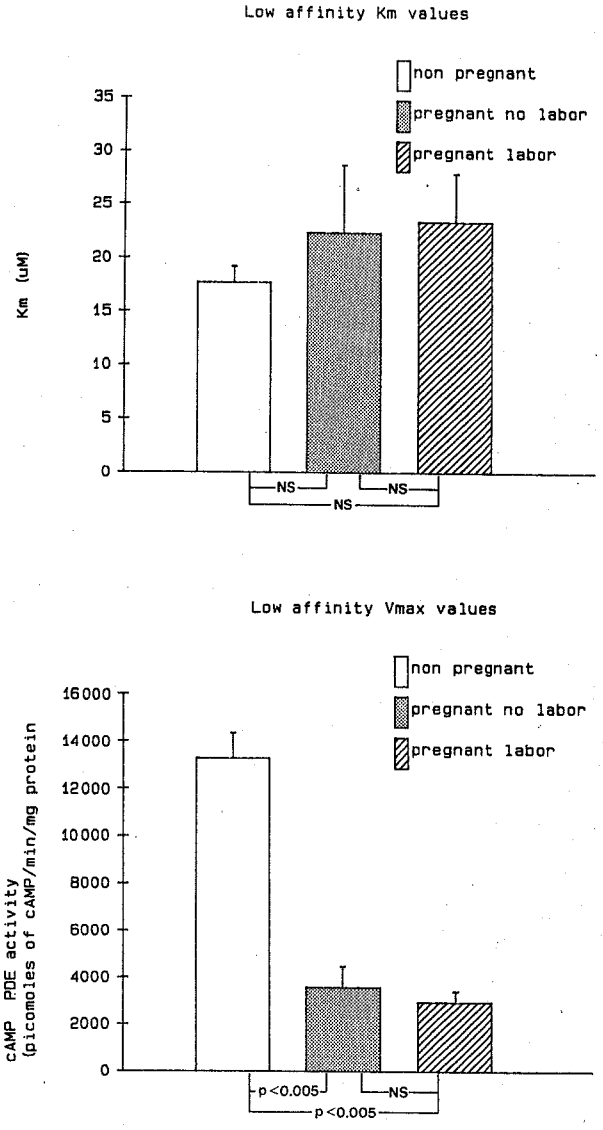

Fig. 3. *Top,* Low affinity Km is not significantly different in the three conditions. *Bottom,* Low affinity maximum velocity during pregnancy is also approximately 75% less than in the nonpregnant state.

in the uterus during the course of pregnancy.[2,3,6,9] Since the intracellular levels of cyclic adenosine monophosphate in part depend on the activity of cyclic adenosine monophosphate–phosphodiesterase, our findings suggest that in normal pregnancy a physiologic condition exists that decreases degradation of cyclic adenosine monophosphate, by inhibition of the activity of cyclic adenosine monophosphate–phosphodiesterase, and thus maintains adequate levels of this vital compound.

Although several investigators have examined cyclic adenosine monophosphate–phosphodiesterase activity in different species, few studies exist that have dealt with human myometrial cyclic adenosine monophosphate–phosphodiesterase.[5,10,11] Published studies of human uterine cyclic adenosine monophosphate–phosphodiesterase are contradictory concerning the kinetic characteristics of the enzyme. One group[5] reported the existence of two enzymes with apparent Km values of 12.6 μmol/L for the high affinity and 91 μmol/L for the low affinity enzyme and another group[11] reported the existence of one enzyme with an apparent Km of 1.56 μmol/L. This discrepancy may be related to differences in methodology, a frequent problem in cyclic adenosine monophosphate–phosphodiesterase research and one that makes comparisons difficult. Leroy et al.[6] reported the existence of two enzymatic forms with apparent Km values of 2.8 μmol/L for the high affinity enzyme and 57 μmol/L for the low affinity enzyme. These values are 2.5 times higher than values we report and this can be explained by the different substrate concentrations used. However, their findings are indicative of the existence of two enzymatic forms and are in agreement with our findings. In addition they resolved two peaks of phosphodiesterase activities on diethylaminoethyl cellulose chromatography. Peak I hydrolyzed both cyclic adenosine monophosphate and cyclic guanosine monophosphate and was activated by the ionized calcium–calmodulin complex and peak II was insensitive to ionized calcium–calmodulin and hydrolyzed only cyclic adenosine monophosphate.

Our findings support the existence of two enzymatic forms of cyclic adenosine monophosphate–phosphodiesterase in human myometrial cytosolic preparations and are also in agreement with animal studies in rats, rabbits, sheep, and primates.[2,12-16] The Km value of the high affinity enzyme in our studies remained fairly constant throughout, from the nonpregnant state to term pregnancy and subsequent labor. The low affinity Km value varied slightly but this change was not significant. However, the maximum velocity of both enzymes exhibited a 75% decrease from the nonpregnant to pregnant state. No significant difference exists in both the Km and maximum velocity values between samples from women in labor or not in labor. The lower activity of phosphodiesterase during pregnancy in comparison to the nonpregnant state may increase cyclic adenosine monophosphate levels and thus contribute to myometrial relaxation and pregnancy maintenance.

During active labor the activity of phosphodiesterase was similar to the activity of the enzyme obtained from women not in labor. The study design does not permit any conclusion regarding the significance of cyclic adenosine monophosphate–phosphodiesterase in the initiation of labor. Whether phosphodiesterase activity increases just before or during the early phase of labor and then returns to prelabor levels is a question that might best be examined in an animal model.

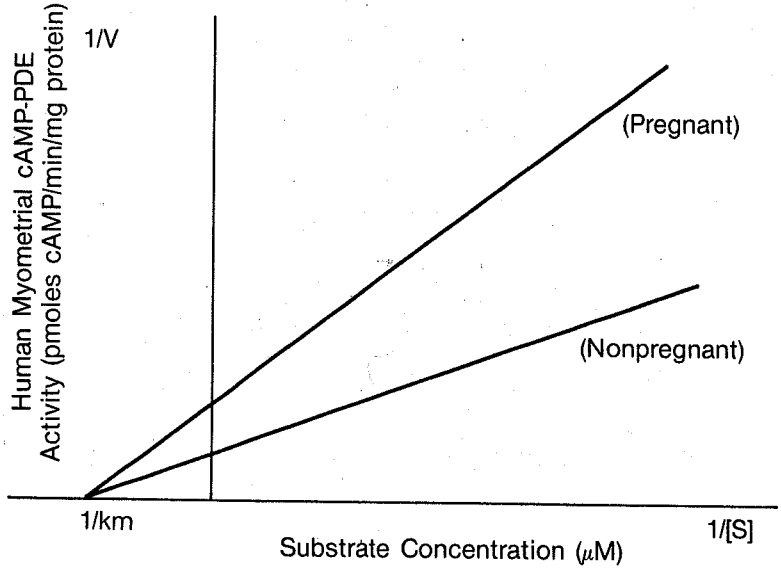

Fig. 4. Noncompetitive inhibition of human myometrial cyclic adenosine monophosphate–phosphodiesterase during pregnancy.

A potential criticism is the fact that the tissue was taken from the lower uterine segment, the least contractile part of the uterus. In one patient (not part of the present data) who underwent lower segment vertical cesarean section with extension to the upper segment, the enzyme activity between upper and lower segment was examined and was not different. In addition, all previous studies in humans examined tissue from the lower segment and this makes the comparisons more meaningful. Berg et al.[11] reported higher phosphodiesterase activity in women treated with terbutaline for preterm labor in comparison to a group of women who were not treated and were not in labor. They concluded that terbutaline was responsible for the increased phosphodiesterase activity, but a comparison between patients in and not in labor may render their conclusion invalid.

Consideration of myometrial phosphodiesterase has potential clinical applications. In the treatment of preterm labor β-agonists are frequently used. However, the use of these potent drugs has been associated with serious cardiopulmonary complications, which are dose related and may be fatal. Several selective phosphodiesterase inhibitors have been developed and are in clinical use as cardiotonic and antiarrhythmic agents.[17, 18] The development of similar selective inhibitors of myometrial phosphodiesterase might help us improve the efficacy of β-mimetic drugs and decrease the dosage required and thus the dose-related complications of these drugs. Conceivably the development of an inhibitor that is specific for myometrial cyclic adenosine monophosphate–phosphodiesterase could eliminate the serious cardiovascular complications associated with increasing levels of cyclic adenosine monophosphate in the myocardium.

In summary, human myometrial cytosol contains two enzymatic forms of cyclic adenosine monophosphate–phosphodiesterase, one low and one high affinity. The Km values, of both enzymes, are similar in the nonpregnant and term pregnant myometrium. However, the maximum velocity of phosphodiesterase in the term pregnant myometrium is 75% less than in the nonpregnant myometrium. Comparison of the enzyme from women in labor with the one from women not in labor did not reveal any differences in both Km and maximum velocity for both enzymatic forms. These findings suggest that cyclic adenosine monophosphate–phosphodiesterase may contribute to myometrial relaxation and pregnancy maintenance.

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