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The Thermodependence of Uterine Adenylate Cyclase in the Rat: Its Response to Fluoride and Decidual Stimulation

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ABSTRACT. The thermodependence of adenylate cyclase (AC) from rat uteri during Day 4 (D4) of early pseudopregnancy after trauma was studied using radiochemical analysis. AC activity, measured at 4-55°C, exhibited maximal activity between 30-35°C. In this temperature range, the enzyme activity varied between 16.60 ± 0.76 and 17.00 ± 0.33 picomoles cAMP per min per mg protein. An Arrhenius plot of the enzyme activity showed a single slope between 4-30°C. The energy of activation (E_a) was 15.94 ± 0.31 Kcal mol^{-1} deg^{-1} and the Q_{10} (20-30) was 2.04 ± 0.02. Incubation of the nontraumatized uteri with 10 m*M* sodium fluoride exhibited a temperature optimum similar to that of the traumatized uteri. In this temperature range, AC activity varied between 18.64 ± 0.50 and 18.39 ± 0.47 picomoles cAMP per min per mg protein. An Arrhenius plot of the AC activity exhibited a single slope between 4-30°C. The Q_{10} (20-30) was 2.38 ± 0.04. These data suggest a similarity between decidual stimulation and sodium fluoride with respect to the activation of uterine adenylate cyclase.

During early progestation, the uterus of the rat becomes transiently sensitive to the blastocyst or to traumatic stimuli (DeFeo 1967, Ledford *et al.* 1976), and the endometrium responds to such stimuli by the production of a decidual cell reaction (DCR). One of the earliest biochemical events associated with the induction of the decidual cell reaction is an increase in uterine adenylate cyclase (AC) activity (30-60 sec; Bekairi *et al.* 1984b) and a dramatic and transient increase in uterine cAMP concentration (Leroy *et al.* 1974, Rankin *et al.* 1977, 1979, 1981, Kennedy 1983), a phenomenon which is thought to be among the primary events required to transduce the decidual stimulus and activate a pattern of uterine metabolism which favors decidual growth.

In an earlier report (Sanders *et al.* 1984), it was suggested that a change in the steroid hormone environment favoring progesterone caused a decrease in the

energy of activation for adenylate cyclase the Q_{10} measured at temperatures between 20-30°C.

In an attempt to study this phenomenon in detail, the present investigation was undertaken to: a) examine the thermodependence of adenylate cyclase in homogenates of uteri before and after trauma, b) determine the effect of sodium fluoride on the enzymic activity of varying incubation temperatures, and c) determine the extent to which changes in activational energies occurred during these different conditions.

Material and Methods

Animals

Adult female Holtzman rats (200-300 g) were caged individually in environmentally controlled qaurters: 23 ± 2°C and a 14L/10D photoperiod of fluorescent illumination with the midpoint of the light phase at 12:00 noon. Food and water were provided *ad libitum*. Reproductive cyclicity was monitored by vaginal smears which were recorded for at least 10 days before animals were used in an experiment. Pseudopregnancy was induced in the rats by stimulation of the uterine cervix on the morning of proestrus and estrus; Day 1 of pseudopregnancy was defined as the first day of leukocytic infiltration in the vaginal smear following estrus.

Tissue Preparation

Uteri were obtained from each of three rats on Day 4 of pseudopregnancy. Rats were weighed and killed by decapitation between 09:00 and 12:00 hr, and their abdominal cavities were immediately opened. Uteri were removed quickly, transferred to an ice-cold plate, allowed to cool, and stripped of adherent fat and mesentery. In a second set of experiments, uteri were 'traumatized' *in situ* by massaging them with a blunt instrument. All subsequent manipulations of the tissue were done at 4°C. Each uterus, including both horns, was weighed on a torsion balance, cut into 0.5 cm segments and placed in a Kontes #22 homogenizing tube containing 5 volumes of cold 50 m*M* Tris-HCl, pH 7.4. The tissue was homogenized in Tris buffer (1 uterus/tube) for ca. 2 min, and assayed as described below.

Adenylate Cyclase Assay

Adenylate cyclase activity was measured, as described previously (Sanders *et al.* 1977, Bekairi *et al.* 1984a), using the rate of [α-^{32}P]ATP conversion to cyclic [^{32}P]AMP. The adenylate cyclase assay mixture contained, in a final volume of 0.2 ml, the following: 15 m*M* Tris-HCl (pH 7.4), 5 m*M* $MgCl_2$, 7.4 m*M* theophylline, 14 m*M* phosphocreatine, 30 μg creatine phosphokinase, 1 m*M* cyclic AMP, 0.08% bovine serum albumin, [α-^{32}P]ATP (1.2×10^6 cpm), and 1 m*M* ATP.

Reaction mixtures were preincubated for 30 seconds before being initiated by adding the homogenate. Incubations were performed for 10 min and the reactions were terminated by the addition of 100 μl of 0.75 m*M* HCl. Labeled cAMP was isolated and measured as described previously (Bekairi *et al.* 1984a). Protein was determined by the method of Sachacterle and Pollack (1973) using bovine serum albumin as a standard. The results were expressed as picomoles cAMP per minute per mg protein. All data are presented as means ± SEM of groups comprised of at least 3 rats/point. Selected comparisons between means were analyzed using student's t-test.

Effect of Temperature

The effect of temperature on the activation of adenylate cyclase was measured using uterine homogenate preparations from Day 4 (before and after trauma, in the presence and absence of 10 m*M* sodium fluoride, respectively). All incubations were for 10 min at selected incubation temperatures between 4°C and 55°C. The data are presented using Arrhenius plots and from these data, activation energies and Q_{10} values were calculated.

Reagents

[α-^{32}P]ATP (specific activity 4.0-40.0 Ci/mmole) was purchased from New England Nuclear, Boston, MA, and [8-^{3}H]adenosine 3′,5′-cyclic monophosphate (specific activity 23 Ci/mmole) was obtained from Schwartz-Mann, Orangeburg, NY. Cyclic [^{3}H]AMP was purified by ion exchange chromatography. The reagents and drugs used were: ATP disodium salt, bovine serum albumin, creatine phosphate, creatine phosphokinase, cyclic AMP, glycylglycine, and anhydrous piperazine (Sigma Chemical Company, St. Louis, MO); alumina activity I (ICN Nutritional Biochemicals, Cleveland, OH); manganese dioxide powder (Mallinckrodt, St. Louis, MO).

Results

The effect of temperature on the activation of adenylate cyclase was measured using uterine homogenate preparations from rats during Day 4 after trauma. In these tissues, the enzyme activity showed a temperature optimum at 30-35°C. In this temperature range, the enzyme activity ranged between 16.60 ± 0.76 and 17.00 ± 0.33 picomoles cAMP per min per mg protein (Fig. 1).

It was reported that an Arrhenius plot of adenylate cyclase activity from uteri during Day 4, before trauma, exhibited two slopes with a discontinuity at 20°C (Sanders *et al.* 1984). The energy of activation (E_a) was 25.4 ± 3.3 Kcal mol^{-1} deg^{-1} below 20°C and 4.1 ± 0.8 Kcal mol^{-1} deg^{-1} for temperatures between 20-36°C (Sanders *et al.* 1984). The Q_{10} (20-30) was 1.2 ± 0.1. However, following

trauma to the uterus on Day 4, the discontinuity disappeared and the plot showed a single slope between 4-30°C. The E_a was 15.94 ± 0.31 Kcal mol^{-1} deg^{-1} and the Q_{10} (20-30) was 2.04 ± 0.02 (Fig. 2, Table 1). These data are in agreement with those reported by Sanders *et al.* (1984).

Incubation of the nontraumatized uteri with 10 m*M* sodium fluoride exhibited a temperature optimum of 30-35°C, which is similar to that of the traumatized uteri in the absence of sodium fluoride. At this temperature range, adenylate cyclase

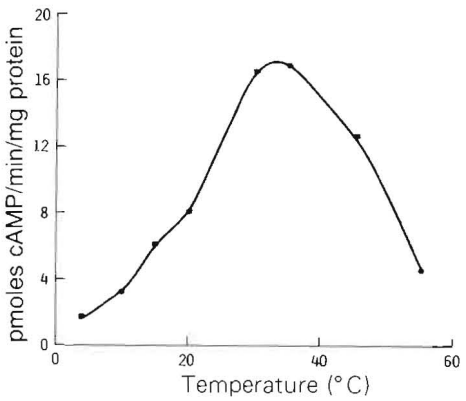

Fig. 1. Thermoregulation of uterine adenylate cyclase activity during Day 4 of progestation following decidual stimulation. Homogenates were prepared as described in Material and Methods. Values are the means of determinations from 3 uteri.

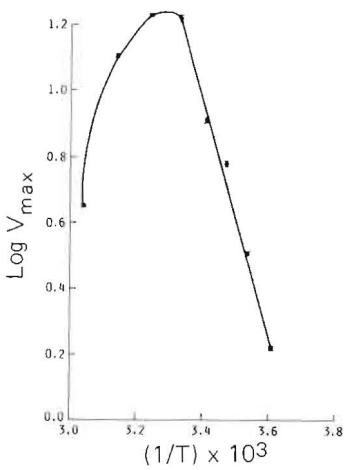

Fig. 2. Arrhenius plot of uterine adenylate cyclase activity from uteri obtained during Day 4 of pseudopregnancy after trauma. Homogenates were prepared as described in Material and Methods. Values are the means of three determinations ± SEM.

Table 1. Energy of Activation (E_a)[a] and Q_{10} (20-30).

Day	4-15°C[b]	20-30°C[c]	4-30°C[d]	Q_{10} (20-30)
D_4 + NaF	18.00 ± 0.9	19.73 ±1.25	16.84 ± 2.38	2.38 ± 0.04
D_4 + trauma	17.97 ± 0.6	17.67 ± 0.3	15.94 ± 0.31	2.04 ± 0.02

[a] (E_a): Energy of activation in Kcal Mol^{-1} deg^{-1}.
[b] (4-15): Energy of activation measured between 4 and 15°C.
[c] (20-30): Energy of activation measured between 20 and 30°C.
[d] (4-30): Energy of activation measured between 4 and 30°C.

activity ranged between 18.64 ± 0.50 and 18.39 ± 0.47 picomoles cAMP per min per mg protein (Fig. 3).

An Arrhenius plot of adenylate cyclase activity, from Day 4 tissue in the presence of 10 m*M* NaF, showed a single slope between 4-30°C (Fig. 4). The E_a was 16.84 ± 0.45 Kcal mol^{-1} deg^{-1} for temperatures between 4-30°C. The Q_{10} (20-30) was 2.38 ± 0.04. These data suggest a similarity between decidual stimulation and sodium fluoride with respect to the thermodependence of uterine adenylate cyclase.

Discussion

The effects of temperature on uterine adenylate cyclase were measured under specified endocrine conditions (Sanders *et al.* 1984). When the logarithms of rates

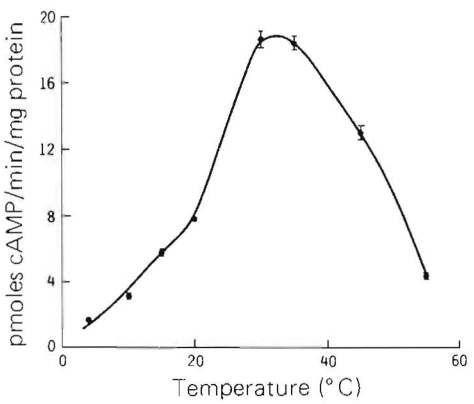

Fig. 3. Effect of incubation temperature on uterine adenylate cyclase activity in the presence of 10 m*M* sodium fluoride during Day 4 of progestation. Homogenates were prepared as described in Material and Methods. Values are the means of three determinations ± SEM.

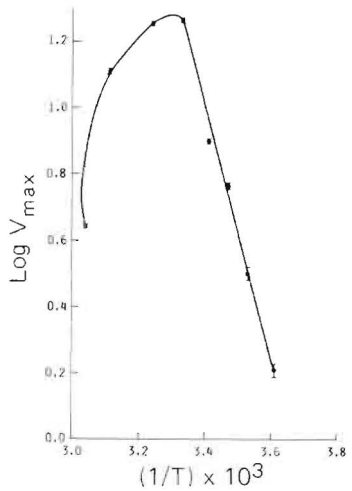

Fig. 4. Thermoregulation of adenylate cyclase activity from uteri obtained during Day 4 of progestation in the presence of 10 m*M* NaF. Homogenates were prepared as described in Material and Methods. Values are the means of determinations from three uteri and are presented in an Arrhenius plot.

of activity were plotted against the reciprocals of the absolute temperature (Arrhenius plots), characteristic inflection points were seen at 20°C (nontraumatized uteri of Day 4 of progestation). The energy of activation (E_a) was shown to be 25.4 ± 3.3 Kcal mol^{-1} deg^{-1} below 20°C and 4.1 ± 0.8 Kcal mol^{-1} deg^{-1} for temperatures between 20-36°C (Sanders *et al.* 1984). The reported Q_{10} (20-30) was 1.2 ± 0.1. The molecular basis of discontinuities in Arrhenius plots cannot be deduced from the studies carried out so far. Dixon and Webb (1979) enumerated several theoretical possibilities. In addition to considering a possible transition (conformational change) of the enzyme itself, one should, in the case of membrane-bound adenylate cyclase, also expect that a phase or structural change of the membrane, of which the enzyme is an integral part, could affect rate parameters and the energy of activation of the overall process. Such a possibility has also been considered by Keirns *et al.* (1973) in a study on the thermodependence of adenylate cyclase from liver. Another explanation for such discontinuity was suggested by Sanders *et al.* (1984). Tissues obtained from animals during Day 4 were exposed to very high *in vivo* concentrations of progesterone, 80 ng/ml, and very low concentrations of estradiol, 15-20 pg/ml (Butcher *et al.* 1975). A change in the steroid hormone environment favoring progesterone caused a decrease in the energy of activation for adenylate cyclase and the Q_{10} measured at temperatures between 20-30°C. Such changes suggested that (a) the membrane surrounding adenylate cyclase might have become more fluid, or (b) uterine adenylate cyclase might exist in two forms, each with a different energy of activation.

Following trauma to the uterus on Day 4 of progestation, the discontinuity disappeared and the plot showed a single slope between 4-30°C. The E_a was 15.94 ± 0.31 Kcal mol^{-1} deg^{-1} and the Q_{10} (20-30) was 2.04 ± 0.02. Day 4 of progestation represents a unique endocrine state in that the uterus can be transiently induced to decidualize by a traumatic stimulus (Yochim and DeFeo 1963). The data in Fig. 2 suggests that following such a stimulus, the Arrhenius plot was no longer discontinuous; the system responded as if it were no longer restricted by membrane configuration. Thus, once activated by trauma, subsequent activation by temperature showed a 'linear' relationship like that for most cytosolic enzymes. If, as suggested by Dixon and Webb (1979), the enzyme exists in two forms, then the activation by trauma on Day 4 resulted in an activation of both forms of adenylate cyclase. If not, a response pattern like that measured during Day 4 before trauma could have been expected.

Incubation of the nontraumatized uteri with 10 m*M* sodium fluoride exhibited a temperature optimum between 30-35°C, which is similar to that of the traumatized uteri in the absence of sodium fluoride. In this temperature range, adenylate cyclase activity ranged between 18.64 ± 0.50 and 18.39 ± 0.47 picomoles cAMP per min per mg protein (Fig. 3). The degree of activation observed in the present study was comparable with that reported for the enzyme of bovine adrenal medulla (Serck-Hanssen *et al.* 1972), but less than that reported for rat kidney and guinea pig heart adenylate cyclase (Forte 1972; Drummond and Duncan 1970).

An Arrhenius plot of uterine adenylate cyclase activity, from tissue during Day 4 in the presence of 10 m*M* NaF, showed a single slope between 4-30°C. The calculated E_a was 16.84 ± 0.45 Kcal mol^{-1} deg^{-1} for temperatures between 4-30°C. The Q_{10} (20-30) was 2.38 ± 0.04. The disappearance of the discontinuity was reported with an adenylate cyclase of higher specific activity obtained from rat brain cortex in the presence of NaF (Bär 1974). Rall and Sutherland (1958) first reported the activation of adenylate cyclase by F^- ions in 1958, but the mechanism of this effect has remained obscure. According to Spiegel and Dawns (1981), F^- can act by inhibiting GTPase activity *via* an interaction with the G-protein in the membrane.

Thus, the results of the present experiments are consistent with a hypothesis which suggests that uterine adenylate cyclase is membrane-bound and may exist in two forms, each with a different energy of activation. A change in the endocrinology which supports progestation results in a decrease in the energy required to activate adenylate cyclase between 20-30°C. Furthermore, the similarity between decidual stimulus and sodium fluoride with respect to the thermodependence of uterine adenylate cyclase suggests that decidual stimulus might act at the level of the regulatory subunit, G/F protein, of uterine adenylate cyclase.

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الاعتماد الحرارى للإنزيم الرحمي أدنيلات سيكلاز فى الفأر: استجابته للفلوريد وتنبيه الغشاء الساقط

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الرياض - المملكة العربية السعودية

تمت دراسة التأثير الحرارى على نشاط الإنزيم أَدِنَيْلات سيكلاز المستخلص من أرحام إناث الفئران فى اليوم الرابع بعد الصدمة الكهربائية فى مرحلة الحمل الكاذب باستخدام التحليل الإشعاعي. عند قياس نشاط الإنزيم بين درجتي حرارة ٤-٥٥°م وجد أن الانزيم يُحقق أعلى نشاط له مابين ٣٠-٣٥°م. فى هذا المدى الحرارى، تراوح نشاط الإنزيم مابين ١٦,٦ ± ٠,٧٦، ١٧,٠٠ ± ٠,٣٣ بيكومول أحادى فوسفات الأدينوسين الحلقى فى الدقيقة/واحد ملِّيجرام بروتين.

أعطى تمثيل نشاط الإنزيم بمنحنى أرهينيوس منحنٍى انفراديًا ما بين ٤ و ٣٠°م. أما طاقة التنشيط فكانت مساوية للقيمة (٢٠-٣٠°م) تساوى ٢,٠٤ ± ٠,٠٢ كما أن تحضين الأرحام غير المصدومة كهربائيا مع ١٠ ميلمول فلوريد الصوديوم أظهر درجات حرارة مشابهة لتلك المتحصل عليها من الأرحام المصدومة كهربائيا. فى هذا المستوى الحرارى، تراوح نشاط إنزيم أدنيلات سيكلاز مابين

١٨,٦٤ ± ٠,٥ و ١٨,٣٩ ± ٠,٤٧ بيكومول أحادى فوسفات الأدينوسين الحلقى فى الدقيقة/واحد ملّيجرام بروتين.

ولقد أظهر رسم أرهينيوس بأن نشاط الإنزيم هو ذو منحنًى انفرادى بين ٤ و ٣٠°م. قيمة درجة الحرارة المطلقة (٢٠-٣٠°م) تساوى ٢,٤٨ ± ٠,٠٤ تبين هذه المعلومات أن مفعـول المؤثر ومفعول فلوريد الصوديوم بالنسبة لنشاط أدنيلات سيكلاز الرحمى متشابهان.