The Effect of Ovarian Hormones on Stress Relaxation in the Uterus of the Pregnant Rat

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Abstract

Tissue strip taken from rat uterine ampulla on day 20 of pregnancy was elongated at a constant rate in Tyrode's solution at 37°C to the length of a specified peak stress, and the stress decline was recorded at this length. The stress decline in uterine strips of ovariectomized progesterone-treated rats was significantly slower than those in intact pregnant rats at any different peak stress and elongation rate examined. Treatment with estradiol as well restored the physical properties to the control levels. Changing the medium to Ca-free Tyrode's solution had no effect on stress relaxation. Results suggest that estrogen with progesterone improves stress relaxation in the rat uterus during late pregnancy by changing the state of the noncontractile elements in the tissue.

In ovariectomized pregnant rats treated with progesterone, intrauterine pressure in late pregnancy is markedly higher than that in intact pregnant rats (Ichikawa and Tamada, 1980), and fetal injury occurs at the critical period when fetuses change from a spheroidal to a cylindrical shape (Tamada and Ichikawa, 1980). Since treatment with estrogen as well prevents the increase in intrauterine pressure and fetal injury, it has been suggested that estrogen may ensure sufficient plasticity of the uterus to allow for the increase in conceptus size at the critical period. Although stress relaxation which is closely related to uterine plasticity has already been examined in the human uterus (Conrad et al., 1966), the effects of ovarian hormones on the physical properties of the uterus have not been studied. The present investigation was undertaken to examine the effect of ovarian hormones on stress relaxation in the uterus of ovariectomized pregnant rats.

Materials and Methods

Animals

Sprague-Dawley rats bred in this laboratory were used. They were maintained at 24±1°C under a lighting schedule of 14 hours light (0500-1900 h). Standard laboratory pellets and water were fed freely. Female rats (60-90 days old) were mated with males at a body weight of 180-220 g. The day when spermatozoa were observed in the vaginal smear was designated as day 1 of pregnancy. The pregnant rats were divided into three groups; one was intact pregnant rats (control rats), and the others were bilaterally ovariectomized on day 14 of pregnancy and treated daily with 4 mg progesterone alone.
(OVX-P rats) or with 4 mg progesterone plus 0.2 μg 17β-estradiol (OVX-PE rats). The steroids were dissolved in 0.2 ml sesame oil and injected subcutaneously at 1600 h each day.

Preparation of the uterine strip

The uterine strips were taken from pregnant rats on day 20 of pregnancy under ether anaesthesia. A uterine strip of 4×25 mm in in situ size was taken along the circular muscle from the antimesometrial side of the middle portion of an ampulla containing a live fetus. In OVX-P rats some fetuses were absorbed or injured, but live fetuses were present in any rats examined. The in situ size of a strip was determined immediately after abdominal incision by placing a calibrated thread on the uterine wall. The length of the uterine strip was marked accurately with suture ties and India ink. The determination was repeated twice or more. The marked area of the uterine wall was then cut off and fixed in in situ solution (NaCl 136.9, KCl 2.7, CaCl2 1.8, MgCl2 1.1, NaHCO3 11.9, NaH2PO4 0.4 and glucose 5.6 in mM). A rectangular strip was made by means of a double-bladed scalpel. The preparation thus obtained was fixed for a distance of 20 mm with a cotton thread to a light metal chain. The weight of the strip was again fixed onto the cork plate in in situ size, and each end of the strip was fixed with a calibrated thread to a light metal chain. The weight of the strip between the two fixed points was calculated by multiplying the measured weight of the strip by 20/25.

Determination of stress relaxation

The experimental arrangement and apparatus used for the determination of stress relaxation were basically similar to those described by Conrad et al. (1966) and Conrad and Ueland (1976). A uterine strip was suspended in a Magnus tube containing 10 ml of aerated Tyrode's solution or Ca-free Tyrode's solution containing 1.0 mM EGTA at 37°C. One chain fixed to the strip was connected to a phosphor bronze cantilever to which strain gauges were firmly fixed, and the other chain was anchored to the bottom of the tube. The signal voltage from the strain gauges was linear with the tension in the chain between 0 and 200 g, although the tension applied in this study was less than 50 g. The cantilever was fixed on the iron table mounted on the rack of a linear head motor (Oriental Service Co., Tokyo). The travel distance of the table was measured with a differential transformer (Shinko Electric Co., Kobe). The travel distance and developed tension of the strip were recorded on a two channel recorder simultaneously. The excursion of the whole system, which was determined by replacing the uterine strip with a stainless steel rod was 0.0178 mm per one gram tension.

After the uterine strip was allowed to equilibrate for 30 min, the motor was turned on to drive the table upward at the rate of 1.5 mm/min until the tension in the strip reached 1 g. The distances between two fixed points on the strip were measured at the time when positive tension appeared and reached 1 g tension utilizing a 10× microscope fitted with an ocular micrometer, and designated as L0 and L0, the basal length of the strip, respectively. The table was turned back to the initial position, and the strip was again elongated at the rate of 1.5 or 6.0 mm/min until the stress reached the specified peak (σmax). Then keeping the strip at the length of peak stress (Lmax), the decline in stress was recorded for 60 min. Since in a preliminary experiment the mean stresses of uterine strips at in situ length were 7.3, 25.9 and 9.2 g/mm² in control and OVX-P and OVX-PE rats, respectively, we took two values of 10 and 40 g/mm² for peak stress; one is near to the value in control and OVX-PE rats and the other is greater than any of the three groups. The length of the uterine strip during determination was computed by adding the basal length (L0) to the travel distance of the table from the point where L0 was determined (Upward=plus, downward=minus), and the value obtained was corrected by subtracting the excursion of the whole system (0.0178 mm/g). The stress was calculated by the following formula.

\[
\sigma = \frac{T}{A_0} - \frac{W}{L_0}
\]

where \( T \) is tension (g), \( A_0 \) is cross sectional area (mm²) at length \( L_0 \), \( W \) is weight (mg) of the strip between the fixed points in the process of elongation, and \( L_0 \) is length (mm) when tension appears.

The rate of stress relaxation (G) was expressed by percentage decline in stress after elongation ceased, and the G value after t min was calculated with the following formula.

\[
G(t) = \frac{\sigma_{max} - \sigma - \sigma_t \times 100}{\sigma_{max}} = \frac{T_{max} - T_{max} 
\]

\[
\sigma = \text{Stress at t min}
\]

\[
T_{max} = \text{Peak tension when elongation was stopped}
\]

When the spontaneous contractions occurred during the determination, values at the baseline were used. Fig. 1 shows a typical experimental result during determination of stress relaxation.

Results

Table 1 shows the ranges of \( L_{max} \) and stress relaxation rates at \( t=60 \) for three experimental rat groups examined under four
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Fig. 1. A typical change in the stress and length of a rat uterine strip during determination of stress relaxation. The rate of stress relaxation \( t \) min after the beginning of stress decline was expressed as the percent decline in stress at \( t \), i.e. \( G(t) = \frac{(\sigma_{\text{max}} - \sigma_t) \times 100}{\sigma_{\text{max}}} \).

Table 1. Length at peak stress and the rate of stress relaxation of uterine strips of progesterone (P) and 17\( \beta \)-estradiol (E\( \beta \))-treated ovariectomized (OVX) pregnant rats.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatments*</th>
<th>No. of rats</th>
<th>Range of ( L_{\text{max}} ) (mm)</th>
<th>( G(60)** ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation rate=1.5 mm/min, peak stress=10 g/mm(^2).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>5</td>
<td>(20.8–21.5)</td>
<td>59.1±0.9(^{ab})</td>
</tr>
<tr>
<td>2</td>
<td>OVX, P</td>
<td>5</td>
<td>(17.4–19.5)</td>
<td>37.0±2.2(^a)</td>
</tr>
<tr>
<td>3</td>
<td>OVX, P+E( \beta )</td>
<td>5</td>
<td>(19.1–20.9)</td>
<td>57.0±1.7(^b)</td>
</tr>
<tr>
<td>Elongation rate=1.5 mm/min, peak stress=10 g/mm(^2), in Ca-free.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>3</td>
<td>(20.5–20.9)</td>
<td>64.2±5.5(^a)</td>
</tr>
<tr>
<td>5</td>
<td>OVX, P</td>
<td>3</td>
<td>(18.8–19.1)</td>
<td>33.2±3.7(^e)</td>
</tr>
<tr>
<td>6</td>
<td>OVX, P+E( \beta )</td>
<td>3</td>
<td>(19.7–20.9)</td>
<td>55.4±0.9(^{be})</td>
</tr>
<tr>
<td>Elongation rate=1.5 mm/min, peak stress=40 g/mm(^2).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>5</td>
<td>(21.5–23.3)</td>
<td>47.7±2.1(^d)</td>
</tr>
<tr>
<td>8</td>
<td>OVX, P</td>
<td>5</td>
<td>(20.3–21.1)</td>
<td>29.1±0.8(^f)</td>
</tr>
<tr>
<td>9</td>
<td>OVX, P+E( \beta )</td>
<td>5</td>
<td>(20.7–23.8)</td>
<td>46.1±1.6(^d)</td>
</tr>
<tr>
<td>Elongation rate=6.0 mm/min, peak stress=40 g/mm(^2).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>3</td>
<td>(20.4–21.8)</td>
<td>50.1±1.0(^{cd})</td>
</tr>
<tr>
<td>11</td>
<td>OVX, P</td>
<td>3</td>
<td>(20.0–20.8)</td>
<td>34.0±2.5(^{ef})</td>
</tr>
<tr>
<td>12</td>
<td>OVX, P+E( \beta )</td>
<td>3</td>
<td>(21.6–22.0)</td>
<td>49.4±1.5(^{cd})</td>
</tr>
</tbody>
</table>

* Control, intact pregnant; OVX, ovariectomized on day 14 of pregnancy; P, 4 mg P daily; P+E\( \beta \), 4 mg P and 0.2 \( \mu \)g E\( \beta \) daily. ** \( G(60) \), percentage decline of stress against peak stress at \( t=60 \), (mean±SE). Means with different alphabetical superscripts in the column of \( G(60) \) differ at a level of \( p<0.05 \) (Duncan's new Multiple Range test).

Different conditions. At a peak stress of 10 g/mm\(^2\) the length \( (L_{\text{max}}) \) of the uterine strips of some rats, especially of OVX-P rats, were less than the \textit{in situ} length of 20 mm. But when the uterine strips were pulled to 40 g/mm\(^2\), \( L_{\text{max}} \) was over 20 mm in all rats.

The replacement of Tyrode’s solution with Ca-free Tyrode’s solution had no effect on stress relaxation, and the mean values for \( G(60) \) determined in the two different solutions were not significantly different in any rat group (groups 1–3 vs. groups 4–6). When
uterine strips were pulled to the peak stress of 40 g/mm² stress relaxation curves became flatter than the curves of strips pulled to 10 g/mm², and mean values of G(60) of strips pulled to 40 g/mm² were smaller than the values of strips pulled to 10 g/mm² in all rat groups (groups 1–3 vs. groups 7–9). When the elongation rate of the uterine strip was increased from 1.5 mm/min to 6.0 mm/min, the decline in stress became faster during the first several minutes, and the mean values for G(6) of the strips pulled at these different rates were significantly different in any rat group. But the differences became smaller after 6 min and no significant difference was observed at t=60 (groups 7–9 vs. groups 10–12).

In any experimental conditions examined, stress relaxation curves in OVX-P rats were always flatter than curves in OVX-PE and control rats (Fig. 2) and mean values for G(60) in OVX-P rats were significantly lower than in any other two rat groups (Table 1). Curves in OVX-PE rats were similar to those in control rats and mean values for G(60) in both groups were not significantly different in any experiment except for the values determined in Ca-free Tyrode’s solution.

Spontaneous contractions occurred in 11 of 39 strips when the stress relaxation was measured in normal Tyrode’s solution. The increases in tension due to contractions were in the range between 0.24 and 1.74 g, and contractions appeared to be simply superimposed on the normal relaxation curve.

Discussion

Harkness and Harkness (1959, 1960) reported that an increase in the extensibility of the collagenous framework of the rat uterine cervix preceded parturition and a further increase was caused in vitro by applying trypsin and chymotrypsin which do not attack collagen. On the basis of these results, they have suggested that the increase in the extensibility of the rat uterine cervix may be a result of the slip of collagen fibrils, which is caused by hydrolysis of the cement substances between collagen fibrils (Harkness and Harkness, 1960). Furthermore, according to Conrad et al. (1966), glycerination or other treatments which enhance contractions of the uterine muscle did not change the stress relaxation in the human uterine muscle, but treatments with trypsin.

Fig. 2. Typical stress relaxation curves of uterine strips of intact pregnant (control), ovariectomized progesterone-treated (OVX-P) and ovariectomized progesterone+estradiol-treated(OVX-PE) rats. The strips were elongated at the rate of 1.5 mm/min to the peak stress of 10 g/mm² in Tyrode’s solution.
or pronase which affect connective tissue elements increased the rate of stress relaxation in the muscle. They concluded that stress relaxation in the uterine muscle depends on connective tissue elements but not primarily on muscular elements. In the present study, suppression of contractile elements by placing the uterine strip in Ca-free Tyrode's solution had no effect on stress relaxation in the muscle, and spontaneous contractions of the uterine muscle did not change the stress relaxation. These results also indicate that contractile elements in the uterine muscle have no effect on stress relaxation.

The stress relaxation in OVX-P rats was slower than that in intact pregnant rats in any elongation rate and peak stress, and \( G(60) \) in OVX-P rats was significantly smaller than that in intact pregnant rats. However, the treatment with estradiol as well restored stress relaxation to the level of intact pregnant rats. These results suggest that estrogen improves stress relaxation of the rat uterus in late pregnancy. The mechanism of the enhancing action of estrogen on stress relaxation is not clear. But estrogen increases the content and metabolic rate of glycosaminoglycans in the uterus (Sinohara and Sky-peck, 1964; Endo and Yosizawa, 1973; Takata and Terayama, 1977), which are main structural elements of proteoglycan aggregate (Rosenberg et al., 1975), and proteoglycan and collagen are main components in extracellular materials surrounding cells and closely associate each other in the extracellular matrix (Mathews, 1965). Then the changes in the state of glycosaminoglycans due to estrogen may alter the physical properties of extracellular materials in the uterine tissue.

Since the material which exhibits stress relaxation is defined as having viscoelastic property, the present results suggest that estrogen together with progesterone may contribute to uterine adaptation to the rapid growth of fetuses during late pregnancy by improving the viscoelastic property of the uterine wall.

As progesterone generally decreases the excitability of the uterine muscle and makes it inactive, the tension of the uterine muscle may also decrease in OVX-P rats. But a marked increase in intrauterine pressure during late pregnancy in OVX-P rats, as has been reported previously (Ichikawa and Tamada, 1980), indicates that in OVX-P rats the increased tension of the connective tissue elements due to estrogen-insufficiency overcomes the dampening effect of progesterone on the muscular elements.

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References


Rosenberg, L., W. Hellmann and A. K. Kleinschmidt

