Length tension relationships in the nonpregnant and pregnant rat uterus and the effect of antiprogestin

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Length–tension relationships and the tissue composition of the corpus and the cervix uteri were investigated in a rat model. Four groups of rats were used: nonpregnant (n = 12); day 18 of pregnancy treated with vehicle (n = 8); day 18 of pregnancy treated with the antiprogestin ZK 98 299 (Onapristone) for 19 h (n = 8); and day 22 of pregnancy during spontaneous labour (n = 8). Increased extensibility and maximal contractility in both corpus and cervix uteri were demonstrated with increased gestational age. The collagen concentration was reduced significantly in corporal preparations from pregnant rats compared with those from nonpregnant rats but not in specimens from the cervix. Antiprogestin treatment tended to increase the contractile ability.

Introduction

Cervical ripening is a prerequisite for normal labour and delivery. Therefore, cervical priming with prostaglandins is useful clinically when labour has to be induced (Karim et al., 1968). However, this treatment fails in some women. Moreover, labour contractions may be induced before cervical priming has been obtained. Therefore, new therapeutic principles, for example the use of antiprogestins, need to be studied in relation to both the cervix and myometrium.

Rat and human myometria are composed mainly of muscular tissue. However, in the proximal cervix, the proportion of muscle comprises 40–50% in rats (Harkness and Harkness, 1959), which is almost double that in women. Thus, cervical smooth muscle could play a significant role during labour in rats.

Several studies investigating the mechanism of labour have used isolated rat uterus in organ baths. Information about the passive and active mechanical properties of the cervix and myometrium, and the effects of pregnancy is a prerequisite for such studies. Length–tension relationships have been investigated in myometrial specimens from pregnant and postpartum rats (Izumi, 1985; Garfield and Beier, 1989), but no data are available on rat cervical smooth muscle, in which only passive mechanical properties have been studied (Williams et al., 1982).

Progesterone appears to restrain the activity of the myometrium during pregnancy (Stys et al., 1978; Kubli-Garfias et al., 1983), and this is the basis of the ‘progesterone-block’ theory (Csapo, 1956). In this theory, antiprogestin acts as a progesterone receptor antagonist and thereby induces delivery in rats (Chwalisz et al., 1986) and other species including humans (Frydman et al., 1992). This treatment seems to enhance responses to agonists like oxytocin in isolated myometrium in late pregnant and postpartum rats (Garfield and Beier, 1989). However, it is not known whether antiprogestins influence the length–tension characteristics, and this impairs the interpretation of these findings. Such knowledge is pertinent for future experiments on the influence of antiprogestin treatment on the functional characteristics of the cervix and myometrium.

The objective of the present study was to investigate the length–tension relationships and collagen content of isolated rat myometrium and cervix in nonpregnant, late pregnant and intrapartum rats, and the influence on these parameters of the antiprogestin ZK 98 299 (Onapristone).

Materials and Methods

Thirty-six female rats of the Wistar strain from Mellegaard Breeding Centre Ltd, Denmark were studied. Twelve rats were virgin nonpregnant (NP group, weight when killed: 280 ± 9 g (mean ± SEM). Twenty-four rats were primigravid. The time from mating to delivery was 21.8 ± 0.4 days (mean ± SD). All rats had free access to food and water, and were housed under constant conditions of room temperature (22°C), air humidity (55%) and light (12 h). The pregnant rats were allocated randomly to three groups: eight rats were treated with 10 mg Onapristone (ZK 98 299) subcutaneously on the day 17 of gestation (+ AP group, mean weight when killed: 325 ± 15 g); eight rats received subcutaneous administration of the vehicle (benzyl benzoate and castor oil) on day 17 of gestation (− AP group, mean weight when killed: 314 ± 6 g); and eight rats continued pregnancy to spontaneous delivery (P group, mean weight when killed: 351 ± 18 g).

Nineteen hours after injection of Onapristone or vehicle, the rats in the + AP and − AP groups were anaeasthetized by an intraperitoneal injection of 5% (w/v) pentobarbital (Abbott), and the uterus and cervix were removed immediately. Separate experiments showed no difference in contractile force of the smooth muscle component whether the animals were killed by a blow to the head or by intraperitoneal injection of pentobarbital.

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In the P group, the rats were killed as soon as the first pup had been delivered. This procedure was chosen to achieve maximal cervical ripening. If we had chosen to kill the rats at a certain time on day 22, the effect of the pup passing through the cervix would have been avoided, but the state of ripening of the cervix uteri in the P group would have varied more. Thus, time of delivery of the first pup of rats differed by 20 h.

After excision, all pericervical tissue was removed. The uterus was fixed by pins in the horns and the cervical lips without stretching the tissue (see Fig. 1). Two transverse specimens of the cervix (a proximal and a distal specimen) were excised at right angles to the long axis of the cervix using a dissecting device consisting of three parallel razor blades mounted 2 mm apart. The most distal razor blade was placed just above the cervical lips, so the two specimens obtained would be from the area where the cervix consists of two parallel connected canals (Fig. 1). From the lateral part of the cervix, two separate, paired preparations were cut (Fig. 2).

Myometrial tissue was achieved as a 2 mm × 15 mm specimen dissected from the antimesenterial part of one of the uterine horns, parallel to the longitudinal axis. The specimen was selected from areas in between implantation sites. From these specimens, the final strips were cut and silk ligatures were tied at each end (2.5–4.0 mm between the knots). On a per animal basis, two strips (left and right side) of the proximal cervix, two strips (left and right side) of the distal cervix and two strips from the myometrium were prepared. Only one set of data from each region (proximal–distal; cervix–corporal) of each animal was obtained, as the other strip was used as a control. One value from each rat was pooled for each region, and the mean is presented (Tables 1–3 and Figs 1–3).

The specimens were immersed immediately in Krebs' solution at 4°C, bubbled with 5% CO₂ in O₂, and kept overnight in the fridge. Separate studies showed that this procedure did not affect contractile responses.

**Biomechanical testing**

The next day, the strips were transferred to thermostatically controlled (37.5 ± 0.5°C) 5 ml organ baths containing Krebs' solution (for composition, see below) bubbled with 5% CO₂ in O₂. The pH of the organ bath fluid was 7.40 ± 0.05 and the solution in the bath was exchanged every 20 min. Measurements of Na⁺ concentration in the Krebs' solution in the organ bath, taken to determine whether significant evaporation occurred, showed no significant difference during this period (Maigaard et al., 1986).

At the start of the length–tension experiment, six strips (two from corpus uteri, two from the proximal and two from the distal part of the cervix uteri) were mounted between two small L-shaped hooks. One hook was attached to a force transducer (Grass FT03) for measuring isometric tension, and the other to a sledge, a moveable device that allowed adjustment of the length of the strip. The specimens from the nonpregnant rats were not analysed blind owing to the different increase in length in these strips compared with preparations from pregnant animals, whereas the other three groups were analysed blind. All rats within the four groups were studied at random.

The strips were stretched to a small load of 0.2 mN, and then relaxed spontaneously to zero level, as defined by the unloaded transducer. The length between the knots (L) and the diameter of the strips were measured. The length–tension experiments were carried out on one of the strips, and the other was used as a paired control. After an equilibration period of at least 1 h, the preparations had developed resting
Table 1. Cross-sectional area (A: mm²) and length (L: μm) of the preparations from nonpregnant (NP) controls (~ AP), rats treated with antiprogesterone (+ AP) and rats in parturition (P)

<table>
<thead>
<tr>
<th></th>
<th>NP (n = 12)</th>
<th>-AP (n = 8)</th>
<th>+AP (n = 8)</th>
<th>P (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus uteri</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₀ (mm²)</td>
<td>2.12 ± 0.18</td>
<td>1.21 ± 0.15</td>
<td>1.56 ± 0.19</td>
<td>1.41 ± 0.25</td>
</tr>
<tr>
<td>L₀ (μm)</td>
<td>1756 ± 158</td>
<td>1913 ± 104</td>
<td>2050 ± 82</td>
<td>2350 ± 174</td>
</tr>
<tr>
<td>L₀/L₀ start</td>
<td>1.31 ± 0.05</td>
<td>1.65 ± 0.08</td>
<td>1.61 ± 0.09</td>
<td>1.60 ± 0.07</td>
</tr>
<tr>
<td>Proximal cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₀ (mm²)</td>
<td>1.58 ± 0.23</td>
<td>1.44 ± 0.22</td>
<td>1.04 ± 0.12</td>
<td>1.41 ± 0.42</td>
</tr>
<tr>
<td>L₀ (μm)</td>
<td>2159 ± 111</td>
<td>2784 ± 130</td>
<td>2630 ± 147</td>
<td>3374 ± 242</td>
</tr>
<tr>
<td>L₀/L₀ start</td>
<td>1.24 ± 0.03</td>
<td>1.44 ± 0.06</td>
<td>1.37 ± 0.04</td>
<td>1.53 ± 0.07</td>
</tr>
<tr>
<td>Distal cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₀ (mm²)</td>
<td>2.19 ± 0.24</td>
<td>1.58 ± 0.33</td>
<td>1.48 ± 0.34</td>
<td>0.79 ± 0.09</td>
</tr>
<tr>
<td>L₀ (μm)</td>
<td>1720 ± 107</td>
<td>2075 ± 223</td>
<td>1925 ± 92</td>
<td>2238 ± 139</td>
</tr>
<tr>
<td>L₀/L₀ start</td>
<td>1.18 ± 0.02</td>
<td>1.39 ± 0.06</td>
<td>1.34 ± 0.06</td>
<td>1.41 ± 0.06</td>
</tr>
</tbody>
</table>

All values are means ± s.e.m.
*P < 0.05 preparation versus corpus uteri, same gestation.
†P < 0.01; ‡P < 0.01; §P < 0.001 preparation versus NP, same location.
*P < 0.05 proximal versus distal preparation, same gestation.
**P < 0.05 preparation versus partus, same location.

Tension. Tension in the control strips was gradually increased to 3 mN, while the length–tension experiment was initiated in the other strip.

During the length–tension-experiments, the following four steps were performed. (i) In the pregnant groups, the preparation was stretched by 10% of L₀ and then 10% of L镽ing, all measured in Ca²⁺-free medium. In the nonpregnant groups, the strips were stretched by 5% and 10%, respectively, of the previous L镽ing. Thus, more readings were obtained per length, but the length at 10% stretching could still be compared with the pregnant groups. Stretch resulted in an increase in tension and then relaxation to a new stable tension (TᵢCa²⁺−free). (ii) The medium was then changed to Ca²⁺-containing Krebs’ solution. A new stable tension (T镽ing) was reached within 10 min. (iii) The medium was changed to 124 mmol K⁺ 1⁻⁻ solution and the maximal tension was measured (T_total). (iv) When the peak of the 124 mmol K⁺ 1⁻⁻-induced contraction had been reached, the medium in the organ baths was changed three times, and the tension decreased to TᵢCa²⁺−free.

Steps (iii–iv) were carried out at each length until two consecutive K⁺-induced contractions showed a decreasing amplitude, thereby defining Lₒ as the length at which optimal/maximal active tension was reached.

Separate experiments showed that responses to 124 mmol K⁺ 1⁻⁻ were similar or higher than responses to prostaglandin F₂α (10⁻¹⁰ mol L⁻¹), oxytocin (10⁻⁸ mol L⁻¹), vasopressin (10⁻⁷ mol L⁻¹), or combinations of these agonists with 124 mmol K⁺ 1⁻⁻ resulted in potentiated responses which, however, showed marked tachyphylaxis. Repeated maximal stimulation may result in irreversible damage of the contractile tissue (Peterson and Paul, 1974), and 124 mmol K⁺ 1⁻⁻ was chosen as the agonist in the length–tension experiments.

The amplitude of the 124 mmol K⁺ 1⁻⁻-induced contraction at Lₒ (Aₒ) was measured in mN per cross-sectional area at Lₒ (Aₒ). Subsequent tension measurements were also related to Aₒ. In control strips, reproducible contractions induced by 124 mmol K⁺ 1⁻⁻ could be achieved for >10 h.

After the length–tension experiments, the strips were weighed, and immediately frozen and kept at ~80°C. The dry weight was measured after freeze-drying; the specimens were hydrolysed in 6.0 mol HCl 1⁻⁻ at 100°C for 16 h and the hydroxyproline concentration was estimated. The concentration of collagen was estimated under the assumption of 13.4% hydroxyproline concentration (Neuman and Logan, 1950). The extractability in acetic acid with pepsin was also estimated in the specimens.

Composition of solutions

Krebs’ solution contained 119 mmol NaCl 1⁻⁻, 4.6 mmol KCl 1⁻⁻, 15 mmol NaHCO₃ 1⁻⁻, 1.5 mmol CaCl₂ 1⁻⁻, 1.2 mmol MgCl₂ 1⁻⁻, 1.2 mmol NaH₂PO₄ 1⁻⁻ and 11 mmol glucose 1⁻⁻.
Calcium-free medium contained 119 mmol NaCl l\(^{-1}\), 4.6 mmol KCl l\(^{-1}\), 15 mmol NaHCO_3 l\(^{-1}\), 1.2 mmol MgCl_2 l\(^{-1}\), 1.2 mmol NaH_2PO_4 l\(^{-1}\), 11 mmol glucose l\(^{-1}\) and 0.01 mmol ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetae (EGTA) l\(^{-1}\).

K\(^+\) solution contained 124 KCl l\(^{-1}\), 15 mmol NaHCO_3 l\(^{-1}\), 1.5 mmol CaCl_2 l\(^{-1}\), 1.2 mmol MgCl_2 l\(^{-1}\), 1.2 mmol NaH_2PO_4 l\(^{-1}\) and 11 mmol glucose l\(^{-1}\).

**Declarations**

The cross-sectional area (\(\mu\text{m}^2\)) was calculated by \(A = \pi r^2\). \(A_{\text{start}}\) and \(L_{\text{start}}\) express the cross-sectional area and the length, respectively, at the beginning of the length–tension experiment. \(L_o\) expresses the length at which maximal active tension \(T_{\text{active}}\) was reached. After this, the second contractions had less amplitude. \(A_0\) expresses the cross-sectional area at \(L_o\).

\[T_{\text{active}} = T_{\text{total}} - T_{\text{resting}}\]

The difference between \(T_{\text{resting}}\) and \(T_{\text{Ca}^{2+}\text{-free}}\) represents \(T_{\text{active(resting)}}\).

**Statistical analyses**

The variances of the length–tension readings differed in the four groups. Therefore, changes from the nonpregnant to the – AP group and finally to the P group were tested by a nonparametric test (Mann–Whitney), \(P\) values were given assuming normal distribution, which was found to be reasonable when drawn. A test probability below 0.05 was considered significant.

Differences between responses in preparations from different locations were calculated by a nonparametric test (Mann–Whitney), since the variance differed among the groups.

Differences between the proximal and distal cervical specimens within each group were tested by a paired \(t\) test. The difference between the proximal and distal specimens was analysed by Kruskal–Wallis test to investigate whether this difference was expressed in a different way in the four groups.

The effect of antiprogestin in the rats at day 18 of pregnancy was analysed by a nonparametric test (Mann–Whitney), because the variance differed between the groups. Differences between the – AP and P groups were also analysed by a Mann–Whitney test.

**Results**

The ratio \(L_o/L_{\text{start}}\) in both corpus and cervix uteri were higher in pregnant rats than in the nonpregnant group, indicating a more marked extensibility (Table 1). In the corporal specimens from the P group, the cross-sectional area was 50% of the initial \(A_{\text{start}}\) value at the length of maximal tension \(L_o\), whereas it was 77% in the NP group. Antiprogestin treatment did not influence these parameters.

Spontaneous activity was observed in all specimens from the corpus uteri in the P and + AG groups, and in 88% and 58% of the specimens in the – AG and NP groups, respectively. Neither amplitude nor frequency of spontaneous activity at \(L_o\) in the corporal specimens (measured over the last 5 min before addition of K\(^+\) solution) differed significantly among the groups.

In the cervical specimens, spontaneous activity was observed after addition of Ca\(^{2+}\)-containing Krebs’ solution in the first 5 min of a new length in the first 2–3 contractions and had always disappeared before \(L_o\) was reached. In specimens from the proximal cervix, spontaneous activity was seen in

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**Table 2. Characterization of corpus, proximal and distal preparations of rat uterus**

<table>
<thead>
<tr>
<th></th>
<th>NP (n = 12) Mean</th>
<th>SEM</th>
<th>- AG (n = 8) Mean</th>
<th>SEM</th>
<th>+ AG (n = 8) Mean</th>
<th>SEM</th>
<th>P (n = 8) Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus uteri</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>1.74</td>
<td>0.16**</td>
<td>0.83</td>
<td>0.10</td>
<td>1.18</td>
<td>0.08</td>
<td>1.46</td>
<td>0.19^6</td>
</tr>
<tr>
<td>Water (%)</td>
<td>84.4</td>
<td>0.6</td>
<td>85.2</td>
<td>0.5</td>
<td>85</td>
<td>0.2</td>
<td>85.4</td>
<td>1</td>
</tr>
<tr>
<td>Coll/dry wt (%)</td>
<td>68.6</td>
<td>3.1***</td>
<td>42.6</td>
<td>4.3</td>
<td>44</td>
<td>5.1</td>
<td>53</td>
<td>2.8^5</td>
</tr>
<tr>
<td>Extrat. (%)</td>
<td>48.2</td>
<td>2.2</td>
<td>47.5</td>
<td>7</td>
<td>48.2</td>
<td>7^7</td>
<td>67.6</td>
<td>1.6^5^5</td>
</tr>
<tr>
<td>Proximal cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>1.13</td>
<td>0.07</td>
<td>1.09</td>
<td>0.09</td>
<td>0.96</td>
<td>0.11</td>
<td>0.85</td>
<td>0.13^5</td>
</tr>
<tr>
<td>Water (%)</td>
<td>82.8</td>
<td>0.5</td>
<td>81.7</td>
<td>2.4</td>
<td>84.8</td>
<td>0.6</td>
<td>84.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Coll/dry wt (%)</td>
<td>60.6</td>
<td>2.9^11</td>
<td>57.1</td>
<td>4.4</td>
<td>54.6</td>
<td>5.7^1</td>
<td>64.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Extrat. (%)</td>
<td>47.2</td>
<td>2.1^12</td>
<td>41.6</td>
<td>7.5</td>
<td>40.4</td>
<td>3.6</td>
<td>59.3</td>
<td>3.8^5</td>
</tr>
<tr>
<td>Distal cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>1.92</td>
<td>0.13**</td>
<td>1.01</td>
<td>0.09</td>
<td>1.03</td>
<td>0.11</td>
<td>0.78</td>
<td>0.15^5</td>
</tr>
<tr>
<td>Water (%)</td>
<td>81.3</td>
<td>2.8</td>
<td>84.5</td>
<td>1.2</td>
<td>85.2</td>
<td>0.9</td>
<td>84.5</td>
<td>1.1^3</td>
</tr>
<tr>
<td>Coll/dry wt (%)</td>
<td>69.4</td>
<td>3.1</td>
<td>64.2</td>
<td>7.3</td>
<td>68.7</td>
<td>7.8</td>
<td>68.9</td>
<td>4</td>
</tr>
<tr>
<td>Extrat. (%)</td>
<td>38.3</td>
<td>2.2</td>
<td>38.7</td>
<td>5.1</td>
<td>43.8</td>
<td>3.8^4</td>
<td>60.6</td>
<td>3.6^5</td>
</tr>
</tbody>
</table>

*\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); preparation versus –AG, same location.
*\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); preparation versus P, same location.
*\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); preparation versus NP, same location.
*\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); proximal versus distal preparation, same gestation.
Coll: collagen concentration; Extract: extractability.
Table 3. Tensions at L₀ for corpus uteri, proximal and distal cervix uteri from rats

<table>
<thead>
<tr>
<th></th>
<th>NP (n = 12)</th>
<th>~ AP (n = 8)</th>
<th>+ AP (n = 8)</th>
<th>P (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{resting}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus</td>
<td>1.90 ± 0.29</td>
<td>2.94 ± 0.65</td>
<td>3.27 ± 0.92</td>
<td>5.51 ± 1.85*</td>
</tr>
<tr>
<td>Proximal</td>
<td>4.87 ± 1.55*</td>
<td>3.53 ± 0.48</td>
<td>4.00 ± 0.69</td>
<td>2.68 ± 0.55</td>
</tr>
<tr>
<td>Distal</td>
<td>5.35 ± 1.14**</td>
<td>2.99 ± 0.89</td>
<td>2.00 ± 0.60±**</td>
<td>3.69 ± 1.78</td>
</tr>
<tr>
<td>T_{active(resting)}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus</td>
<td>—</td>
<td></td>
<td>0.88 ± 0.74</td>
<td>1.08 ± 0.88</td>
</tr>
<tr>
<td>Proximal</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_{active}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus</td>
<td>5.23 ± 0.57</td>
<td>23.14 ± 6.62†</td>
<td>34.94 ± 4.46††</td>
<td>45.34 ± 8.86††</td>
</tr>
<tr>
<td>Proximal</td>
<td>7.07 ± 1.08‡</td>
<td>10.85 ± 1.20†</td>
<td>12.54 ± 1.50†***</td>
<td>14.14 ± 1.85††***</td>
</tr>
<tr>
<td>Distal</td>
<td>3.06 ± 0.57**</td>
<td>7.76 ± 1.51*</td>
<td>7.71 ± 1.92***</td>
<td>11.96 ± 1.86††***</td>
</tr>
<tr>
<td>T_{total}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus</td>
<td>7.14 ± 0.68</td>
<td>26.08 ± 6.38††</td>
<td>38.20 ± 4.70††</td>
<td>50.85 ± 10.21††</td>
</tr>
<tr>
<td>Proximal</td>
<td>11.84 ± 2.18</td>
<td>14.37 ± 1.34‡</td>
<td>16.54 ± 1.80**</td>
<td>16.83 ± 2.14**</td>
</tr>
<tr>
<td>Distal</td>
<td>8.41 ± 1.48</td>
<td>10.75 ± 1.92*</td>
<td>9.53 ± 2.37**</td>
<td>16.73 ± 2.92**</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

Table 3: tension of the preparation in Krebs' solution at L₀; T_{active(resting)}: difference in tension from Ca²⁺-free to Krebs' solution at L₀. T_{active}: T_{active(resting)} - T_{resting}; T_{total}: maximal tension in the preparation in 124 mmol K⁺ 1⁻¹ solution at L₀.

*P < 0.05; **P < 0.01 preparation versus corpus uteri, same gestation.
†P < 0.05. ‡P < 0.01. ††P < 0.001 preparation versus NP, same location.
*P < 0.05 proximal versus distal preparation, same gestation.

Collagen concentration

The collagen concentration (in relation to dry weight) in the pregnant rats was significantly higher in the distal cervix than in the corpus (Table 2). Collagen concentrations were similar in corpus and cervix in the NP group. The collagen concentration in corporal preparations was significantly lower in pregnant rats compared with rats in the NP group. Extractability was increased in the P group compared with the NP group for all three locations.

Length tension relationships

Differences due to location. The tension levels at L₀ are shown (Table 3) and force per cross-sectional area A₀ is shown in relation to UL₀, where the curves have been centered on L₀ (Fig. 3).

In corporal preparations, T_{active(resting)} was observed only in + AP and P rats, where it reached about 2% of T_{active} (Table 3, Fig. 3). T_{active} was two- to threefold higher in corporal than in cervical specimens, except in the NP group.

In cervical preparations, T_{active(resting)} was not observed. T_{active} and T_{total} tended to be higher in the proximal compared with the distal part of the cervix, but this reached significance only for T_{active} in NP group and T_{total} in the ~ AP group (Table 3). T_{active} and T_{total} were higher in the corpus than in the cervix in all pregnant rats.

Effects of pregnancy and early labour. T_{active} at L₀ in corporal strips was increased in the pregnant groups compared with the NP group, with a maximum in the P group (Table 3, Fig. 3). Thus, T_{active} at L₀ increased fourfold over the first 18 days of gestation, and doubled again over the last 4 days before delivery.

T_{active} at L₀ in the cervix was at least doubled from the NP group to the P group (Table 3). In general, T_{active} in the distal cervix was lower than that in the proximal part but, in both locations, an increase in T_{active} was seen from day 18 until early labour (Table 3).

Effects of antiprogestin treatment. Antiprogestin treatment at day 18 of gestation appeared to induce an approximately 50% increase in T_{active} but the difference did not reach significance. The treatment did not affect the other length-tension parameters.

Discussion

The present study demonstrated a marked increase in myometrial contractile ability during pregnancy and early labour, as reflected by an almost exponential increase in T_{active}. This finding is in accordance with previous studies in rats (Izumi, 1985), ewes (Stys et al., 1978) and women (Tumbling and Anderson, 1971). Garfield and Beier (1989) found a lower amplitude of KCl-induced contractions in term pregnant than preterm rat myometrium, in contrast to the present finding of an almost twofold increase during this period. However, in the present study, assessment of length-tension relationships was performed to allow for standardized comparisons, and late pregnancy seemed to imply a further increase in the maximum myometrial contractile potential.
The increased response to KCl depolarization may be caused by hyperplasia, that is, an increased number of cells per cross-sectional area, by cellular hypertrophy or by increased contractile ability of the individual muscle cell secondary to, for example, increased sensitivity towards calcium. If the increased contractile ability was caused by hyperplasia, the number of muscle cells per cross-sectional area in the corpus should be nine times the number in the nonpregnant uterus (for details, see appendix). This is unlikely, since the separate muscle cell cross-sectional area would then have to be reduced. This leaves hypertrophy or increased contractile ability for the individual muscle cell as possible explanations for the increased $T_{\text{active}}$ at term.

Several morphological studies indicate some degree of cellular hypertrophy during pregnancy (Hegele-Hartung et al., 1989; Word et al., 1993). However, there is also reason to believe that changes in excitation–contraction coupling and calcium sensitivity of the contractile proteins also contribute to enhanced cellular contractile ability. Thus, saponin-skinned rat myometrium shows enhanced calcium sensitivity with increased gestational age (Izumi, 1985), and an increased number of Ca$^{2+}$ and Na$^{+}$ membrane channels (Sperlakis et al., 1992).

Analysis of collagen content showed a decrease in the corporal specimens in pregnancy compared with the nonpregnant state, whereas no major changes were found in the cervical specimens. This is similar to the results of Williams et al. (1982), but in contrast to the lower content of cervical collagen at term seen in women (Uldbjerg, 1989). However, changes in the functional characteristics of the cervical collagen seem to be induced at term, as indicated by a higher extractability.

Progesterone restrains gap junction formation and myometrial contractile activity during pregnancy in sheep and rats (Garfield et al., 1980; Kubli-Carfas et al., 1983). This finding supports the early ‘progesterone-block’ theory (Capo, 1956) and forms the rationale for the development of antiprogestins. These drugs enhance uterine sensitivity to contractile agonists (Bygdeman and Swann, 1985; Elger et al., 1987; Bygdeman et al., 1991).

In the present study, antiprogestin treatment failed to affect cervical and myometrial length–tension relationships significantly, although a tendency towards increased contractility was seen, in agreement with the study of Garfield and Beier (1989). However, in a previous study, significant changes in cervical mechanical properties were observed (Raadestad, 1993), which is consistent with the established use for induction of abortion when used in combination with prostaglandins. However, the present study indicated that the cervical and myometrial effects of this treatment is less marked than the changes induced by the endogenous priming process and early labour.

In conclusion, the present study defined length–tension relationships for myometrial and cervical uterine tissues from pregnant and nonpregnant rats as of importance for standardized studies of smooth muscle activity in this area. The results support the evidence that cellular hypertrophy, together with
enhanced sensitivity to agonists and calcium, contributes to the enhanced myometrial contractility at term.

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Appendix

Smooth muscle cells are normally arranged in parallel strings of serial connected cells. The number of muscle cells per cross-sectional area should be known so that the contractility in different preparations can be compared. This could not be assessed in the present study, since analysis of collagen content was performed. However, a cautious estimate may be obtained as follows:

The connection between the cross-sectional area A of a preparation and the mean number of muscle cells in the area A (m) is:

$$m = A \cdot \rho$$

where \( \rho \) is the number of muscle cells per cross-sectional area.

When \( Q \) is defined as the increase in the mean contractility per muscle cell, the following equation is valid:

$$T_{active} = \rho \cdot Q$$

Therefore:

$$\frac{T_{active}^P}{T_{NP active}^P} = \frac{Q_{active}^P}{Q_{NP active}^P} \cdot \frac{p^P}{\rho^P}$$

$$\downarrow$$

$$\frac{Q_{active}^P}{Q_{NP active}^P} = \frac{T_{active}^P}{T_{NP active}^P} \cdot \frac{p^P}{\rho^P}$$

where \( P \) denotes values from rats at delivery and \( NP \) values from nonpregnant rats.

If the values for \( T_{active} \) from the corpus are introduced (Table 3):

and

$$Q_{active}^P > Q_{NP active}^P$$

It seems unlikely that the density of muscle cells (\( \rho \)) for the \( P \) group should be approximately nine times higher than for the \( NP \) group. Therefore:

$$Q_{active}^P > Q_{NP active}^P$$