97. Relationship between Morphological Change and Electrical Activity of Longitudinal Muscle Layer of the Rat Myometrium Following Ovarietomy and Hormonal Treatment

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Changes in electrical property of the rat myometrium following hormonal treatment have been investigated by Kuriyama and Suzuki (1976). They described that in both normal and spayed rats, oestradiol hyperpolarized the membrane and generated a burst of spikes on a sustained depolarization. On the other hand, progesterone slightly hyperpolarized the membrane and generated burst discharges without a sustained depolarization. Simultaneous treatment with progesterone and oestradiol produced a plateau potential of long duration during burst discharges. When the passive electrical properties of the membrane were compared, the length constant of the tissue was increased by treatment with oestradiol, and it was further increased by simultaneous treatments with oestradiol and progesterone. However, the length constant of the tissue was reduced by treatment with progesterone.

To investigate further the effects of progesterone and oestradiol on the spayed rat myometrium, changes in the morphological arrangement of the tissue were examined in relation to the changes in the passive membrane properties.

The rats of Wister-King variety were used. Virgin female rats (180–230 g and 2–3 months old) were anesthetized with nembutal (30 mg/kg; sodium pentobirbital, Abbott Lab.) and picrotoxin (1 mg/kg; Wako Pure Chem. Ind., Ltd.) by intraperitoneal injection and ovarietomized. Seven to ten days after ovarietomy, either progesterone (10 mg/day; synthetic progesterone, Machida Pharm. Co., Ltd.), oestradiol (10 µg/day oestradiol-17 benzoate, Teikokuzoki, Pharm. Co., Ltd.) or both simultaneously was injected subcutaneously. The following symbols define the hormone treatment; P5 is 5 days treatment with progesterone, E5 is 5 days treatment with oestradiol and EP5 is 5 days simultaneous treatment with oestradiol and progesterone.

For electron microscopical experiments, rats were stunned and
bled, then the uterus was dissected out quickly. The horn of the uterus was fixed in 3% buffered glutaraldehyde at 0–5°C for 30 min, then sectioned into small pieces less than 1 mm and rinsed with buffer. The pieces were fixed again in 2% osmium tetraoxide for 2 hr at 0–5°C. They were subsequently dehydrated through a graded series of alcohol and embedded in epoxy resin (Epon 812). All tissues prepared for electron-microscopic observation were double stained with lead and uranyl acetate, and examined. To measure the cell to cell connection in spayed and hormone treated myometria, the tissue was cut transversely and close appositions between the cells were calculated from 10 to 15 nonoverlapping electron-micrographs from one section of individual tissues.

The negative (5 or 6,000×) was enlarged at 44,000 times by photoinstrumentation. To identify the cell connection as nexuses, gap junctions, or so on, the negatives were further enlarged to higher magnification (80,000×), if they showed a five-lined (nexus or gap junction) or a seven-lined (intermediate contact, interdigitation or

Table I. The sizes of the cell (A) and numbers of cell to cell connection (B) measured from castrated and hormone treated rat myometria

<table>
<thead>
<tr>
<th></th>
<th>Castrated</th>
<th>E5</th>
<th>P5</th>
<th>EP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum size</td>
<td>15.95</td>
<td>32.08</td>
<td>8.90</td>
<td>29.64</td>
</tr>
<tr>
<td>Minimum size</td>
<td>0.25</td>
<td>0.57</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean size</td>
<td>3.54</td>
<td>8.82</td>
<td>1.39</td>
<td>6.09</td>
</tr>
<tr>
<td>S. E.</td>
<td>0.25</td>
<td>0.42</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>Measured number</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

The negative was measured at p<0.001 p<0.001 p<0.001

<table>
<thead>
<tr>
<th></th>
<th>Castrated</th>
<th>E5</th>
<th>P5</th>
<th>EP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum number</td>
<td>9</td>
<td>8</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Minimum number</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean number</td>
<td>2.9</td>
<td>2.7</td>
<td>4.1</td>
<td>3.5</td>
</tr>
<tr>
<td>S. E.</td>
<td>0.13</td>
<td>0.12</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>Measured number</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

The tissue samples were obtained from longitudinal muscle layer. Individual numbers of pieces of tissue examined for castrated. Oestradiol treated (E5), progesterone treated (P5) and oestradiol with progesterone treated (EP5) were 6–10, and 200 electron micrographs were measured from the individual tissues. The size and number were measured from electron micrographs of smooth muscle cells in transverse orientation.

As shown in Table IA, the mean size of cells was increased to more than twice in E5 (2.5 times) and it was reduced to less than one half in P5 (0.38 times) compared to that observed in spayed ones (p<0.001). The mean size of cells in EP5 was also increased (1.7 times; p<0.001) but it was less than that measured in E5.

These results clearly indicate that oestradiol produces hyperplasia of the myometrial cell and progesterone produces hypoplasia than the size of spayed ones. These results agreed with the changes in the length constant of the tissue measured under hormonal treatment, i.e. the length constant of the tissue increased in oestradiol treated (from 1.3 mm to 2.5 mm in E5) and oestradiol with progesterone treated (to 2.6 mm in EP5) rats. However, this value was reduced in progesterone treated rats (to 1.0 mm in P5) (Kuriyama and Suzuki, 1976).

From the cable theory, length constant of the tissue (\(\lambda\)) could be expressed as follow; 
\[
\lambda = \sqrt{\frac{a}{2}} \sqrt{\frac{r_m}{r_i}}
\]
(Hodgkin and Rushton, 1946; Katz, 1948), where \(a\) is radius, and \(r_m\) and \(r_i\) are membrane resistance and internal resistance, respectively. Therefore, factors modifying the length constant of the tissue besides the radius of the cell are membrane resistance and internal resistance of the tissue. Internal resistance was composed of myoplasmic resistance and the resistance produced by cell to cell connection (Tomita, 1970). The types of cell to cell connections in the mammalian smooth muscle have been classified from the morphological findings into nexus, gap junction, intermediate contact, interdigitation and simple apposition. Many investigators have thought that these arrangements possess low resistance path between cells (Henderson, 1975). However, in the present experiments, neither nexus nor gap junction between the cells in both longitudinal and circular muscle was elucidated except at the last stage of gestation, and most types of cell to cell contact were simple opposition, intermediate contact and interdigitation. The number of above structures per single cell was increased by treatment with progesterone or by simultaneous treatment with progesterone and oestradiol as compared with that observed in spayed rat, while no obvious change in number was observed by treatment with oestradiol alone (Table IB, p<0.30).

Recently, Garfield et al. (1977) reported that in the sections of longitudinal and circular muscle of myometrium obtained from rats during pregnancy, at term, during delivery and post partum, gap junction were only recognized during or immediately prior to delivery
and post partum. The absence of gap junction was also observed in the spayed and hormone treated rats. The present results confirmed the above observations. Garfield et al. (1977) postulated that no gap junction during early and mid-pregnancy might correspond to a phenomenon described as ‘defense mechanism of pregnancy’ to maintain the pregnancy until the term (Csapo, 1961).

The length constant of the tissue, however, can only be calculated when the cell possess cable-like property by their connections as a functional bundle. The present results, therefore, might indicate that the increased cell to cell connections in progesterone treated rats might produce the reduction in the internal membrane resistance, but this reduction contributes only a minor role to determining of the length constant of the tissue. Presumably, formation of gap junction or nexus might produce a larger reduction in the internal resistance. On the other hand, in oestradiol treated rats, the number of cell to cell contact per single cell was not increased, whereas the length constant was increased.

The change in the internal structures of myometrium after treatment with oestradiol was characterized by increase in ribosomes, rough endoplasmic reticulum and Golgi complex, and also the size of mitochondria was slightly enlarged without increase in numbers. However, progesterone did not produce any remarkable changes in the internal structure of the cell. These results agreed with those described on oestradiol treated rat by Bo et al. (1968) and Ross and Klebanoff (1967), and also with the biochemical observations done on glycogen contents and RNA synthesis in oestradiol treated myometrium (Noteboon and Gorski, 1963). Bo et al. (1968) have described that numbers of inpocketing or caveolae distributed on the surface membrane were decreased by treatment with oestradiol. However, the present experiments failed to confirm the above observations, and the changes of membrane structure and thickness by treatment with oestradiol or progesterone were not observed.

The obtained results might indicate that electrical connections between the cells to constitute the cable property in rat myometrium are not due to the nexus structure, and either close appositions between the cells or undetectable structure by electronmicroscopic method at the present procedure contribute them. The present experiments strongly suggest that the visible numbers of cell to cell contact obtained by the electronmicroscopic technique do not seem to play an essential role to determine the passive electrical property of smooth muscle cell membranes of the rat myometrium following ovariectomy and hormonal treatments.
References

444–479.
250–255.