INVolvement of the uterine blood vessels in the refractory state of the uterine stroma which follows oestrogen stimulation in progesterone-treated mice

L. Martin, R. C. Hallowes, C. A. Finn and D. G. West

Departments of Hormone Physiology and Pathology, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX and Royal Veterinary College, Royal College Street, London, NW1 OTU

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summary

Increases in uterine capillary permeability after the injection of oestradiol into spayed mice, appeared to be caused by the development of vascular fenestrations and not by the separation of adjoined endothelial cells. Progesterone did not prevent the uterine weight, oedema and vascular responses to the first of two injections of oestradiol but inhibited those to the second. It was concluded that the failure of repeated oestrogen-treatment to produce uterine oedema in progesterone-treated mice resulted from the refractory state which develops after the first injection and which extends to the endometrial vasculature.

Introduction

Oestradiol stimulates uterine stromal proliferation in mice pretreated with progesterone (Martin & Finn, 1968, 1970). The stroma then becomes refractory, as further doses of oestrogen fail to induce further stromal proliferation (Finn, Martin & Carter, 1969; Martin & Finn, 1971). They also fail to produce the massive uterine oedema characteristic of repeated treatment with oestrogen alone (Martin & Finn, 1970). However, it was not known if this failure to produce oedema was due to the refractory state, or simply to an anti-oedema effect of progesterone. Accordingly we studied the effects of progesterone on uterine weight increases produced during the first 6 h after the injection of oestrogen, and which have been shown to be almost entirely due to increase in water content (Astwood, 1938; Ham, Hurley, Lopata & Ryan, 1970). In the rat this increased water content is associated with the development of transient gaps in the endothelial lining of capillaries and small venules (Friederici, 1967; Ham et al. 1970). We examined the ultrastructure of the stromal vasculature of the mouse uterus to determine whether an absence of oedema in progesterone-treated mice was due to a failure of the vessels to develop these gaps.
ANIMALS AND METHODS

Seven-week-old albino mice weighing 23–25 g were anaesthetized with Avertin (tribromoethyl alcohol, Bayer Products Co.) and ovariectomized by a dorsal incision. Eight days later the mice were divided into two groups. Mice in the control group received five daily doses of 0.05 ml arachis oil, those in the other group received five daily doses of 1 mg progesterone. The groups were then divided into four sub-groups. Mice in the first received 50 ng oestradiol 6 h before killing, those in the second, 50 ng oestradiol 30 h before killing, those in the third, 50 ng oestradiol both 30 and 6 h before killing and those in the last 0.05 ml arachis oil.

The animals were killed by cervical dislocation. The uteri were dissected free of fat, blotted on filter paper, weighed on a torsion balance, and fixed at constant length (2.5 cm) in Bouin’s fluid; 5 μm transverse sections were prepared from the mid-region of each uterus and stained with Cole’s haematoxylin and eosin. The presence or absence of oedema was evaluated subjectively. The experiment was replicated four times and involved a total of 156 mice.

For electron microscopy the peritoneal cavity was opened and flooded with cold 3% glutaraldehyde in Sorensen’s 0.1 M-phosphate buffer, pH 7.4. The uteri were rapidly dissected and pinned to a constant length of 2.5 cm in ice-cold fixative, where they remained for 2–3 min. An 0.5 cm length from each mid-region was cut transversely into 0.1–0.2 cm pieces which were transferred to fresh fixative for a further 3 h. They were then washed by constant agitation in phosphate buffer for 18 h. Subsequently they were cut transversely into 70 μm slices, refixed in 1% buffered osmic acid, dehydrated through graded ethanol and acetone, and random slices were embedded in Araldite (Hallowes & Streek, 1970). After polymerization, slices were chosen at random and trimmed to produce blocks for ultra-thin sectioning on an LKB ultratome. The blocks were taken from the anti-mesometrial pole of the uterus and included endometrium, glands, stroma and either circular or both muscle coats. Ultra-thin sections, 60–80 nm thick, were picked up on copper grids with 45 μm square holes and approximately 50% transmission. Sections were stained with uranyl acetate and lead citrate and examined in a Hitachi 7S electron microscope. Grids were scanned at 3000× and all blood vessels noted. Using the ×7 binocular viewing microscope, it was possible to determine both the type of vessels and the presence of fenestrations. The small vessels were characterized as capillaries, venules or lymphatics according to Ham & Leeson (1965). A fenestration (Plate 1) was defined as a ‘hole’ in the endothelial cell cytoplasm approximately 65 nm wide with an electron-dense membrane 1.6 nm thick separating the vessel lumen from the surrounding ground substance (Rhodin, 1962; Elfvin, 1965; Friedericci, 1968). A vessel was classed as fenestrated if in section it contained one or more fenestrations as defined above. Because of the limitations imposed by electron-microscopical techniques, only a small area of each uterus was examined, but apart from locating each block in the anti-mesometrial region, the selection of tissue was completely random. The choice of slice and block area was made solely on the basis of suitability for ultramicrotomy and neither the user of the ultramicrotome (D. G. W.) nor the electron microscopist (R. C. H.) knew the treatment schedule. A total of 14 mice were used in this part of the study.
**RESULTS**

**Uterine weights and light microscopical appearance**

The increase in uterine weight 6 h after the first injection of oestrogen was greater in the progesterone-treated mice than in the control mice (28 versus 19 mg; Table 1). The uteri of both groups showed stromal oedema. Twenty-four hours later uterine weight had increased by an additional 14 mg in control mice, but had decreased slightly in progesterone-treated mice. At this stage little stromal oedema was seen in either group. In controls, the second injection of oestradiol produced a much greater increase in uterine weight than the first (50 versus 19 mg) and the uteri showed massive oedema. In progesterone-treated mice the second injection produced a smaller increase in uterine weight than the first (16 versus 28 mg) and the uteri showed little oedema.

*Table 1. The effects of progesterone on 6-h uterine weight increases and vascular fenestration in mice receiving one or two injections of oestradiol*

(Mice were killed at 16.00 h on day 5. Uterine wts are means with 18–20 animals/group; counts of fenestrated vessels come from groups of 1–2 mice. A = 0·05 ml arachis oil; P = 1 mg progesterone; Oe = 50 ng oestradiol.)

<table>
<thead>
<tr>
<th>Hormones given at 10.00 h on days:</th>
<th>Hours after oestrigen (mg)</th>
<th>Mean uterine wt ± S.E.M. (mg)</th>
<th>No. of mice</th>
<th>Total Capillaries % Fenestrated</th>
<th>Total Venules % Fenestrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>A</td>
<td>—</td>
<td>36 ± 1·3</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>Oe</td>
<td>6</td>
<td>55 ± 3·5</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>Oe</td>
<td>A</td>
<td>30</td>
<td>69 ± 3·2</td>
<td>1</td>
<td>14</td>
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<tr>
<td>Oe</td>
<td>Oe</td>
<td>30</td>
<td>6</td>
<td>119 ± 7·1</td>
<td>2</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>30</td>
<td>54 ± 2·7</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>P</td>
<td>PoE</td>
<td>6</td>
<td>82 ± 3·4</td>
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<td>43</td>
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<tr>
<td>P oE</td>
<td>P oE</td>
<td>30</td>
<td>79 ± 3·4</td>
<td>1</td>
<td>12</td>
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<tr>
<td>P oE</td>
<td>P oE</td>
<td>30</td>
<td>95 ± 2·9</td>
<td>2</td>
<td>57</td>
</tr>
</tbody>
</table>

**Summary of analyses of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Uterine weights</th>
<th>Fenestrated vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>Mean square</td>
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<tr>
<td>Progesterone (P)</td>
<td>1</td>
<td>249,736</td>
</tr>
<tr>
<td>6 h v. 30 h (T)</td>
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<td>4,480,964</td>
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<tr>
<td>Oestrigen (O)</td>
<td>1</td>
<td>3,257,556</td>
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<tr>
<td>P × T</td>
<td>1</td>
<td>829,728</td>
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<tr>
<td>P × O</td>
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<tr>
<td>T × O</td>
<td>1</td>
<td>85,563</td>
</tr>
<tr>
<td>P × T × O</td>
<td>1</td>
<td>480,267</td>
</tr>
<tr>
<td>Replicates (R)</td>
<td>3</td>
<td>1,194,321</td>
</tr>
<tr>
<td>Replicate interactions</td>
<td>12</td>
<td>90,114</td>
</tr>
<tr>
<td>R × T × O × P</td>
<td>9</td>
<td>52,813</td>
</tr>
<tr>
<td>Within group (error)</td>
<td>124</td>
<td>23,660</td>
</tr>
</tbody>
</table>

* 0·01 < P < 0·05; ** 0·001 < P < 0·01; *** P < 0·001.
† Since the 3rd order interactions were significant (0·01 < P < 0·05) in terms of within-group variability, their mean square was used to compute F values for the main effects and lower-order interactions.
This variation in weight response to oestrogen with progesterone treatment and with number of injections of oestrogen (Table 1, the interaction PTO) was significant (0.01 < P < 0.05).

Ultrastructure of the uterine vessels

Capillaries and venules were present in sections from all uteri. They were most numerous in the stroma and least numerous in the myometrium. Veins were occasionally seen in the stroma and myometrium but arterioles were only seen in the myometrium. Fenestrations were observed in stromal capillaries but not in stromal veins, lymphatics or arterioles. Due to trimming of blocks before sectioning our material contained little myometrium. Of the few myometrial vessels examined, none had fenestrations. The data in Table 1 come entirely from stromal vessels.

Few fenestrated vessels were seen in untreated control animals and none were seen in progesterone-treated animals not treated with oestrogen. Six hours after oestrogen treatment, the proportion of fenestrated capillaries rose to 13% and 17% in control and progesterone-treated animals, respectively. There were no comparable increases in the proportion of fenestrated venules. Twenty-four hours later the proportion of fenestrated vessels had fallen towards control levels in both groups (Table 1 and Plate 2).

In the control group there was a massive increase in the proportion of fenestrated capillaries and of fenestrated venules, 6 h after the second injection of oestrogen. Both types of vessel were enlarged but there was no sign of the intercellular gap described by Friederici (1967) and by Ham et al. (1970). On the contrary, well-defined intact tight junctions were commonly observed in close proximity to fenestrated portions of the endothelial cells (Plate 1). In progesterone-treated mice the second
injection of oestrogen increased the proportion of fenestrated vessels but the increases were much smaller than those in control animals (Table 1). The patterns of response resembled those for uterine weight: the first injection of oestrogen produced bigger increments in progesterone-treated than in control animals, whereas the second injection produced smaller increments in progesterone-treated than in control animals (Text-fig. 1).

**DISCUSSION**

Since pretreatment with progesterone did not prevent the early weight increase and oedema produced by a single injection of oestrogen, the failure of repeated oestrogen treatment to produce oedema in progestin-treated mice (Martin & Finn, 1970) must stem from the refractory state which develops after the initial oestrogen treatment. When two injections of oestrogen were given, the second produced a larger response than the first in control animals but a smaller one in progesterone-treated animals.

The vascular changes paralleled the changes in weight and in the intensity of oedema; fewer vessels developed fenestrations after the second injection of oestrogen in progesterone-treated mice than in controls, whereas more did so after the first injection. It is not clear whether the decreased response was due to insensitivity of the vessels themselves or was secondary to stromal insensitivity.

The fenestrations described were identical in form and dimension with those described in the rat adrenal (Elfvin, 1965), the mouse kidney (Rhodin, 1962; Friedericci, 1968) and other sites at which there is much capillary filtration.

Friedericci (1967) who examined only the myometrial vessels, and Ham et al. (1970), have suggested that separation of intercellular junctions accounted for the increased capillary permeability observed in the rat uterus after oestrogen treatment. We were unable to find such gaps in stromal vessels. Even when oedema was maximal and fenestrations were frequent, junctions between endothelial cells showed no signs of separation. Such separation occurs in response to histamine (Majno & Palade, 1961), in acute inflammation caused by a variety of agents (Majno, 1965; Cotran, 1965; Ham & Hurley, 1965, 1968) and after tissue injury (Cotran & Majno, 1964; Hurley, Ham & Ryan, 1967a, b; Cotran, 1967; Hurley & Edwards, 1969). In this sense it is a non-specific response. Moreover, gaps of this type would not provide specificity of filtration at the molecular level and it seems unlikely that a structure as ill-defined as the basement membrane would do so.

Although the function of increased uterine capillary permeability is not known, it appears to be an integral part of uterine growth, under specific hormonal control and seems to bear a precise temporal relationship to epithelial and stromal proliferation. The close correlation between uterine oedema and vascular fenestration with a number of different hormone regimes indicates that in the mouse at least, these fenestrations provide the basis for increased uterine permeability. Also, as suggested by Elfvin (1965) the structure of fenestrations would provide the means for specificity and selectivity of filtration at the molecular level.
REFERENCES


DESCRIPTION OF PLATES

PLATE 1

Stromal venule from a mouse not pretreated with progesterone and killed 6 h after the second of two injections of 50 ng oestradiol. Regions of the endothelial cytoplasm are attenuated and show numerous fenestrations of which three are marked →. Two tight junctions (T) are visible in close proximity to fenestrated regions.

PLATE 2

Stromal venule from a mouse not pretreated with progesterone and killed 30 h after a single injection of oestradiol. Three tight junctions (T) are visible but there are no fenestrations.