Activity of enzymes involved in pyrimidine nucleotide synthesis during uterine growth in cyclic rats

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Summary. The pyrimidine nucleotide salvage enzymes, uridine and thymidine kinases, and the enzyme, aspartate carbamyltransferase, involved in de novo synthesis, were investigated to assess their relative contributions to the RNA and DNA precursor requirements of uterine growth in cyclic rats. Only aspartate carbamyltransferase reflected the known fluctuations in plasma oestradiol-17β and uterine mitotic activity, being minimal at dioestrous 1 and maximal at pro-oestrus. Treatment of ovariectomized rats with oestradiol-17β stimulated carbamyltransferase activity, but cycloheximide prevented this response, indicating the association between the enzyme and this hormone. Uridine kinase activity did not vary during the oestrous cycle, but there were peaks of thymidine kinase activity on the days of pro-oestrus and the 1st day of dioestrus.

Introduction

Many factors have been investigated in relation to uterine growth in normal cyclic and in oestradiol-treated immature or ovariectomized rats. Those related to the availability of oestrogen are: uptake of water, small molecules, thymidine and uridine; synthesis of protein, RNA and DNA (e.g. see Mueller, 1965; Szego, 1965; Hamilton, Widnell & Tata, 1968; Billig, Barbiroli & Smellie, 1969a, b; Gorski, 1973; Leake, McNeill & Black, 1975); and mitotic rate (Marcus, 1974).

The pyrimidine precursor supplies essential to the increased RNA and DNA synthesis are derived from the diet, salvage of nucleic acid catabolites and by synthesis from HCO₃⁻, glutamine and ATP. This study was undertaken to assess the possible contributions of the salvage (uridine and thymidine kinases) and the de novo synthetic (aspartate carbamyltransferase) pathways to the pyrimidine precursor requirements for uterine growth in cyclic rats. Aspartate carbamyltransferase is the second enzyme in the de novo pathway, and was chosen for study instead of carbamyl phosphate synthetase, the first and control enzyme, because (1) the enzymes are part of a multienzyme complex (Tatibana & Shigesada, 1972) and undergo parallel increases in mitotically stimulated lymphocytes (Ito & Uchida, 1973); (2) the aspartate carbamyltransferase:carbamyl phosphate synthetase ratio remains constant throughout their purification (Mori & Tatibana, 1975); and (3) the instability of carbamyl phosphate synthetase necessitates the addition of several stabilizers, even to crude extracts, and its assay is long and tedious compared with that for aspartate carbamyltransferase (Tatibana & Shigesada, 1972).

Materials and Methods

Animals and treatment

Female Sprague–Dawley rats (190–225 g) were housed in groups of 5 in stainless-steel cages with corn cob bedding in a controlled environment (24°C, 40% humidity and a 12-h photoperiod). Purina laboratory chow and water were always available. At least two complete cycles were observed before the animals were killed on different days of the oestrous cycle, determined by vaginal smears taken daily between 08:00 and 09:00 h. Some rats were killed 5 days after bilateral ovariectomy and 20 h after treatment with a single subcutaneous injection of 10 µg oestradiol-17β (Sigma Chemical Co., St

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Statistical Tucker for volume cals rats 0-1 (1969). The modified Orangeburg of tubes The ment lumen 12-ml effect was greater, basis weight (oestrus), Only weight -28mM-stocksolution)or24mM-[14C]uridine(145 values the substrate reaction was increased 68%. Protein differences were assessed by method of analysis and radioactive T. Randall & Tucker (1969). Except for the substrate, the assays for both kinases are similar (Manson et al., 1976). The substrates used were [14C]-labelled compounds instead of the 3H compounds used by Ives et al. (1969). The reaction was carried out at 38°C and the final concentrations of the reactants were: 5 mm-Tris–HCl buffer (pH 8); 2-5 mm-MgCl2; 5 mm-[14C]thymidine (175 000 c.p.m./0-25 ml 1-28 mm-stock solution) or 24 mm-[14C]uridine (145 000 c.p.m./0-025 ml 1-92 mm-stock solution) and 0-1 ml supernatant or homogenizing buffer in the blank. Protein values were determined by a slight modification of the method of Lowry, Rosebrough, Farr & Randall (1951).

**Statistical analysis**

Differences between means (± s.e.m.) were assessed by analysis of variance or Student’s t test. P values greater than 0-05 were considered insignificant.

**Results**

Only rats with 4-day oestrous cycles were used and days of the oestrous cycle were designated O (oestrus), P (pro-oestrus), D1 and D2 (dioestrus). As expected (Astwood, 1939), uterine wet and dry weight on Days P and O were significantly greater than on Days D1 and D2 (Table 1).

The relative changes in the activities of all enzymes were similar whether expressed on the basis of wet tissue wt or supernatant protein content, and therefore the activities are expressed on the latter basis only. Uridine kinase activity did not alter during the cycle, but thymidine kinase activity was greater on Days D1 and P (Table 1). Aspartate carbamyltransferase activity also changed significantly during the cycle (Table 1).

The results of the experiments with ovariectomized rats are shown in Table 2. Oestradiol treatment caused an increase in uterine wet and dry weight and in aspartate carbamyltransferase activity, which was similar to that of cyclic rats on Day P (P > 0-05). Treatment with cycloheximide alone had no effect on the uterine wet weight or the enzyme activity, but when given with oestradiol the uterine wet weight increased by 68%. This weight gain was largely due to water uptake (Table 2, Exp. 2).
Table 1. Mean ± s.e.m. changes in body weight, uterine wet and dry weight and uridine kinase, thymidine kinase and aspartate carbamyltransferase (ACTase) activities during the oestrous cycle of the rat (6/group)

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>P</th>
<th>O</th>
<th>D1</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>211 ± 6.1</td>
<td>203 ± 5.1</td>
<td>198 ± 3.6</td>
<td>212 ± 6.1</td>
</tr>
<tr>
<td>Uterine wet wt (mg)</td>
<td>339 ± 20.5a</td>
<td>337 ± 15.6a</td>
<td>231 ± 15.6b</td>
<td>251 ± 14.1b</td>
</tr>
<tr>
<td>Uterine dry wt (mg)</td>
<td>58.1 ± 3.0a</td>
<td>60.4 ± 2.7a</td>
<td>45.5 ± 1.9b</td>
<td>47.7 ± 2.9b</td>
</tr>
<tr>
<td>Uridine kinase</td>
<td>35.5 ± 2.1</td>
<td>34.8 ± 1.3</td>
<td>38.5 ± 2.1</td>
<td>38.3 ± 2.5</td>
</tr>
<tr>
<td>(pmol UMP/h/μg protein)</td>
<td>1.66 ± 0.11a</td>
<td>0.94 ± 0.10b</td>
<td>1.70 ± 0.13a</td>
<td>0.77 ± 0.04b</td>
</tr>
<tr>
<td>Thymidine kinase</td>
<td>637 ± 17a</td>
<td>507 ± 23b</td>
<td>350 ± 27c</td>
<td>459 ± 21b</td>
</tr>
<tr>
<td>(pmol TMP/h/μg protein)</td>
<td>60.3 ± 8a</td>
<td>60.4 ± 7b</td>
<td>59.7 ± 8c</td>
<td>59.8 ± 8d</td>
</tr>
<tr>
<td>ACTase</td>
<td>637 ± 17a</td>
<td>507 ± 23b</td>
<td>350 ± 27c</td>
<td>459 ± 21b</td>
</tr>
<tr>
<td>(pmol CA/h/μg protein)</td>
<td>60.3 ± 8a</td>
<td>60.4 ± 7b</td>
<td>59.7 ± 8c</td>
<td>59.8 ± 8d</td>
</tr>
</tbody>
</table>

UMP = uridine monophosphate; TMP = thymidine monophosphate; CA = carbamylaspartate.
Values within rows with different superscripts are significantly different, P < 0.05.

Table 2. Mean ± s.e.m. values for aspartate carbamyltransferase (ACTase) activity and uterine water content of ovariectomized rats treated with 10 μg oestradiol-17β and/or 20 mg cycloheximide/kg body wt

<table>
<thead>
<tr>
<th>Group</th>
<th>Cottonseed oil</th>
<th>Oestradiol</th>
<th>Oestradiol + Cycloheximide</th>
<th>Cycloheximide</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>ACTase (pmol CA/h/μg protein)</td>
<td>479 ± 31a</td>
<td>603 ± 21b</td>
<td>401 ± 20a</td>
<td>428 ± 34a</td>
</tr>
<tr>
<td>Uterine wet wt (mg)</td>
<td>150 ± 10a</td>
<td>252 ± 17b</td>
<td>267 ± 26b</td>
<td>165 ± 7a</td>
</tr>
</tbody>
</table>

Experiment 2

| No. of rats | 4 | 4 | 4 |
| Uterine wet wt (mg) | 175 ± 9.7a | 290 ± 10.6b | 264 ± 28.6b |
| Uterine dry wt (mg) | 35 ± 1.8a | 49 ± 1.5b | 38 ± 2.6b |
| Water content (mg) | 140 ± 7.9a | 241 ± 9.1b | 226 ± 26.0b |
| Water (%) | 79.6 | 83 | 85.3 |

CA = carbamylaspartate.
Values within rows with different superscripts are significantly different, P < 0.05.

Discussion

These results show that the activity of aspartate carbamyltransferase only (de novo pathway) reflects the reported changes in plasma oestriadiol-17β concentrations during the oestrous cycle of the rat (Brown-Grant, Exley & Naftolin, 1970). The oestradiol-17β peak found by Brown-Grant et al. (1970) occurred just before 08:00 h on Day P when our rats were killed and the enzyme activity was highest. The significant drop in aspartate carbamyltransferase activity on Day O coincides with a drop in the plasma oestradiol-17β level to less than 20% of its peak value (Brown-Grant et al., 1970). That the increase in aspartate carbamyltransferase is oestradiol-dependent is apparent from the results of the experiments with ovariectomized rats in which cycloheximide given 1 h before the oestradiol-17β completely inhibited the hormonally induced enzyme elevation (Table 2).

The first peak (Day D1) in thymidine kinase activity (Table 1) occurred when plasma oestradiol-17β concentrations are lowest. This peak coincides with the time when glandular mitosis is maximal (Marcus, 1974). Another possibility is that the increase in thymidine kinase activity on Day D1 may be
concerned with the recycling of thymidine generated by endometrial resorption. The second peak on Day P coincides with the peaks of plasma oestradiol-17β values and the aspartate carbamyltransferase activity and is most probably associated with the concurrent peak of mitosis and uterine growth (Marcus, 1974). In oestrogen-treated immature rats both uterine thymidine kinase activity and thymidine incorporation into DNA are increased (Leake et al., 1975).

That oestradiol-17β administration to immature and/or ovariectomized rats enhances uterine uridine uptake and incorporation into RNA has been amply documented (Billing et al., 1968a; Hamilton et al., 1968; Means & Hamilton, 1966). Uridine kinase activity (Table 1), however, appears not to fluctuate significantly during the normal oestrous cycle of the rat. Studies on cultured hamster V-19 lung cells have shown that uridine kinase is present in excess throughout the cell cycle and, at concentrations in the medium of up to 100 µM, virtually all the uridine entering the cells was phosphorylated (Stambrook, Sisken & Ebert, 1973). A similar excess of uridine kinase in rat uterine tissue could explain its constant values throughout the oestrous cycle. Rat epididymal uridine kinase also remains unchanged after efferent duct ligation and castration, procedures which significantly reduce the aspartate carbamyltransferase activity (Leung & Nishikawara, 1977).

Early effects of oestradiol on the uterus include histamine release, hyperaemia and increased uptake of water and small molecules (Szego, 1965). In immature rats, prior administration of actinomycin D inhibited the effect of oestradiol on RNA synthesis by 85% but uridine uptake, although reduced, still increased 212% (Billing et al., 1969a). The data in Table 2 show that the effect of oestradiol on macromolecular synthesis can apparently be dissociated from its effects on the uptake of water and small molecules.

This work was supported by NIH Grant 1-RO-1 HD-05040.

References


Received 4 January 1978