PROGESTERONE AND OESTRADIOL RECEPTORS IN THE UTERUS OF OESTRADIOL-PRIMED FETAL AND NEWBORN GUINEA-PIGS

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SUMMARY

The responsiveness of the uterus of the guinea-pig to oestrogen treatment was studied in the fetal and perinatal periods. Twenty-four hours after one dose of 1 mg oestradiol/kg body wt to the pregnant guinea-pig, there was no significant increase in uterine wet weight of the fetus but a sevenfold increase in the concentration of progesterone receptors. In the perinatal period, doses of 1, 10 and 100 µg oestradiol led to as much as an 80% increase in uterine wet weight after 24 h in both 2- and 7-day-old guinea-pigs. On the other hand, levels of progesterone receptors in newborn animals showed a smaller increase (twofold) than that which occurred in the fetal uterus. In both fetal and newborn guinea-pigs, total oestradiol-receptor concentrations (both available and occupied binding sites) decreased significantly after treatment with oestradiol. It was concluded that the hormonal effect of oestradiol on progesterone-receptor synthesis can be expressed in the fetus and to an even greater extent than in the perinatal period over the same period of time. In the fetus, this response can be distinguished from the overall uterotrophic effect of oestradiol.

INTRODUCTION

Studies on the developmental aspects of the mechanism of the action of steroid hormones in this laboratory have led to the finding that specific binding sites for various steroid hormones are present in the guinea-pig fetus, i.e. aldosterone in the kidney and oestradiol in the uterus (Pasqualini, Sumida, Gelly & Nguyen, 1977). Moreover, specific binding of oestradiol has been observed not only in the uterus but in various other fetal tissues (Pasqualini, Sumida, Gelly & Nguyen, 1976). In the fetal guinea-pig uterus oestradiol receptors, which can be detected as early as 34–35 days of gestation, increase during fetal development and decrease after birth (Pasqualini et al. 1976). Progesterone binding, however, can only be detected many days after oestradiol receptors are found in the fetal uterus (Pasqualini & Nguyen, 1979a). A causal relationship has been postulated between the oestradiol and progesterone receptors in the fetal uterus under normal physiological conditions and the possible responsiveness of progesterone receptors to oestradiol-priming has been studied. The aim of the present study was to compare the progesterone-receptor concentrations in the guinea-pig uterus before and after birth and the changes occurring after treatment with oestradiol.

MATERIALS AND METHODS

Animals and treatment

Pregnant Hartley albino guinea-pigs and newborn female guinea-pigs were obtained from a commercial breeder and the gestational age of the fetuses was calculated by comparing the
average weights of the fetuses of a litter with a growth curve (Huggett & Widdas, 1951) which had been previously confirmed (Pasqualini et al. 1976). Animals were anaesthetized with ether and the fetuses obtained by laparotomy. Uteri were excised, stripped of adhering fat and weighed.

In the studies involving oestradiol-priming of the animals, pregnant guinea-pigs received 1 mg oestradiol/kg body weight per day (Steraloids, Paris, France) in 1 ml 40% ethanol–saline solution subcutaneously for 1–3 days (control animals received vehicle alone) and animals were killed 1 day after the last injection. The newborn guinea-pigs received one dose only of 1, 10 or 100 µg oestradiol in 0.5 ml 5% ethanol–saline subcutaneously, preliminary experiments having revealed that this treatment was sufficient to elicit the maximum response on progesterone receptors in the fetuses. The same treatment was used in both newborn animals and in fetuses for comparison.

Radioactive steroids

[6,7-3H]Oestradiol (50 Ci/mmol), [17α-methyl-3H]17α,21-dimethyl-19-nor-pregnane-4,9-diene-3,20-dione (R5020; 55.4 Ci/mmol), synthetic R5020 and [1,2-3H]progesterone (56 Ci/mmol) were obtained from NEN Chemicals, Frankfurt, West Germany. Purity was checked by paper chromatography and found to be greater than 95%.

Cell fractionation

All procedures were carried out at 0–4 °C. Uterine tissue (100 mg) was homogenized in 1 ml buffer of 0.01 M-Tris, 1.5 mM-EDTA, 0.5 mM-dithiothreitol, pH 7.4 (TED) in a Teflon–glass Potter–Elvehjem homogenizer. The homogenate was centrifuged at 900 g for 10 min. This operation was repeated three times and all supernatant fractions were pooled and centrifuged at 200 000 g for 30 min to obtain a clear cytosol supernatant fluid containing approximately 1 g protein/l. The nuclear-myofibrillar pellet was extracted three times with 1 ml 0.6 M-KCl in TED buffer, pH 8.5, and all supernatant fractions and the remaining pellet were pooled and centrifuged at 200 000 g for 30 min, giving a clear 0.6 M-KCl nuclear extract. The remaining pellet was precipitated in 5% trichloroacetic acid for DNA measurement by the method of Burton (1956). Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951).

Determination of specific binding of [3H]oestradiol

Specific [3H]oestradiol binding was determined in protamine-sulphate precipitates of the cytosol fraction and 0.6 M-KCl nuclear extract which was diluted three times (Chamness, Huff & McGuire, 1976; Zava, Harrington & McGuire, 1976; Sumida & Pasqualini, 1979a). The pellets were incubated with 1 × 10^-8 M-[3H]oestradiol with and without a 100-fold molar excess of unlabelled oestradiol to measure non-saturable binding. Specific binding was considered to be the difference between the total binding and the non-saturable binding. In this study, the results are expressed as the sum of available and occupied oestradiol-binding sites in the cytosol fraction and the nuclear extract. Binding sites were determined by incubating the cytosol protamine-sulphate precipitates at 30 °C overnight and the nuclear-extract precipitates at 0–4 °C overnight followed by an incubation at 37 °C for 3 h.

Determination of specific binding of [3H]progesterone and [3H]R5020

The cytosol fraction or 0.6 M-KCl nuclear extract was incubated with 3 × 10^-9 M-[3H]progesterone or [3H]R5020 with and without a 100-fold molar excess of unlabelled steroid to measure non-saturable binding. Incubations were carried out at 0–4 °C for 18 h, during which binding of both available binding sites and those sites occupied by endogenous progesterone occurs since exchange is complete at 4 °C over this time period (C. Sumida, unpublished observations). Bound and unbound steroids were separated by adding 1 vol.
dextran-coated charcoal (final concentration, 0.25% charcoal) and 0.025% dextran (both from Sigma, St Louis, U.S.A.) for 10 min at 0-4 °C.

Measurement of radioactivity
Radioactivity was counted in Scintex (Isotec, France) in a Nuclear Chicago liquid scintillation spectrometer Isocap 300 with an efficiency of 43% for tritium.

RESULTS
Effect of administration of oestradiol on wet weight of fetal and newborn guinea-pig uterus
To study the uterotrophic effect of oestradiol in the fetuses, preliminary experiments had been carried out to determine whether transplacental passage of oestradiol into the fetus could occur by administering oestradiol to the mother. It was observed that 30 min after a subcutaneous injection of 50 µCi [3H]oestradiol to the mother, 0.8–1.2% of the radioactivity administered was found in each fetus of which 10–15% was unmetabolized oestradiol (S. Chaudhuri & J. R. Pasqualini, unpublished observation). After this observation, a dose of 1 mg oestradiol/kg body wt was injected subcutaneously for either 1 day or for 3 consecutive days to the mother and the stimulation of uterine wet weight in the fetus was measured 24 h after the last injection. (This dose was found to be the optimal concentration required for the study of the progesterone–receptor response.) One dose of oestradiol over a period of 24 h did not significantly increase uterine wet weight in the fetus but treatment for 3 consecutive days led to a significant increase in weight.

To compare these results on the effect of oestradiol in intra-uterine life with the uterotrophic response postnatally, 1- and 6-day-old guinea-pigs were also treated with three different doses of oestradiol or saline for 24 h. Unlike the fetal uterus which did not respond over 24 h, the wet weights of neonatal uteri increased significantly (Table 1). In the 2-day-old animals, the increase was even greater with a dose of 100 µg than with 1 µg but in the 7-day-old animals this stimulation appeared to have already reached a maximum with 1 µg after 24 h.

Table 1. Effect of various doses of oestradiol given at 1 and 6 days of age on the wet weight of the uteri of newborn guinea-pigs when samples were taken the next day. (Values are means ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Age at time of sampling (days)</th>
<th>Dose of oestradiol (µg)</th>
<th>Wet wt of uterus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Vehicle only</td>
<td>137 ± 17</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>198 ± 24</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>225 ± 28</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>245 ± 36</td>
</tr>
<tr>
<td>7</td>
<td>Vehicle only</td>
<td>141 ± 15</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>248 ± 20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>250 ± 33</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>254 ± 12</td>
</tr>
</tbody>
</table>

Effect of oestradiol treatment on the concentration of progesterone receptors in the uterus before and after birth
The concentration of progesterone receptors was measured in the cytosol fraction and the 0.6 M-KCl nuclear extract by incubation with both [3H]progesterone and [3H]R5020 at 4 °C (3 × 10⁻⁹ mol radioactive steroid/1 with and without a 100-fold molar excess of unlabelled steroid). As indicated in Table 2, a single maternal treatment of 1 mg oestradiol/kg body wt...
was sufficient to increase the concentration of cytosol and nuclear progesterone receptors by seven times in the fetal uterus. In newborn animals, the basal value of the concentrations of progesterone and R5020 receptors was twice as high as in the fetus but various single doses of oestradiol only led to a two- to threefold increase in progesterone and R5020 receptors above control values. It is interesting to note that the proportion of progesterone receptors found in the nuclear extract increased slightly in the uteri of oestradiol-treated fetuses and to more than twice the control values in the uteri of newborn animals.

Table 2. Effect of oestradiol (OE₂) on the concentration of progesterone and R5020* receptors and of total oestadiol receptors (available and occupied) in the uterus of the guinea-pig before and after birth when treatment was given 1 day before the animals were killed. (Values are means±s.d. of duplicate determinations of five to seven experiments and quadruplicate determinations of nuclear binding of [³H]oestradiol; number of animals was six except where shown in parentheses)

<table>
<thead>
<tr>
<th>Age at time of sampling and treatment</th>
<th>[³H]R5020 binding (pmol/mg DNA)</th>
<th>[³H]Progesterone binding (pmol/mg DNA)</th>
<th>[³H]Oestradiol binding (pmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytosol</td>
<td>Nucleus</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Fetuses (54–65 days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle only (10)</td>
<td>3.29 ± 0.15</td>
<td>3.48 ± 0.15</td>
<td>1.34 ± 0.07</td>
</tr>
<tr>
<td>1 mg OE₂/kg body wt to mother (16)</td>
<td>19.91 ± 9.05</td>
<td>3.07 ± 1.79</td>
<td>9.49 ± 4.82</td>
</tr>
<tr>
<td>2 Days old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle only</td>
<td>6.82 ± 0.94</td>
<td>0.48 ± 0.15</td>
<td>2.96 ± 0.44</td>
</tr>
<tr>
<td>1 µg OE₂</td>
<td>11.77 ± 1.86</td>
<td>1.58 ± 0.20</td>
<td>6.94 ± 1.73</td>
</tr>
<tr>
<td>10 µg OE₂</td>
<td>12.35 ± 0.93</td>
<td>2.48 ± 0.25</td>
<td>7.21 ± 0.90</td>
</tr>
<tr>
<td>100 µg OE₂</td>
<td>10.95 ± 2.45</td>
<td>2.26 ± 0.45</td>
<td>8.13 ± 1.94</td>
</tr>
<tr>
<td>7 Days old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle only</td>
<td>5.62 ± 1.88</td>
<td>0.56 ± 0.22</td>
<td>2.28 ± 0.98</td>
</tr>
<tr>
<td>1 µg OE₂</td>
<td>8.41 ± 1.34</td>
<td>2.18 ± 0.45</td>
<td>5.11 ± 1.72</td>
</tr>
<tr>
<td>10 µg OE₂</td>
<td>9.41 ± 0.88</td>
<td>3.33 ± 0.81</td>
<td>6.11 ± 0.64</td>
</tr>
<tr>
<td>100 µg OE₂</td>
<td>10.75 ± 0.61</td>
<td>2.63 ± 0.57</td>
<td>6.24 ± 0.37</td>
</tr>
</tbody>
</table>


Effect of oestradiol treatment on the concentration of oestradiol receptors in the uterus before and after birth

To see whether there was any correlation between the levels of oestradiol receptors and the increased concentrations of progesterone receptors found 24 h after a single dose of oestradiol, the specific binding of oestradiol (the sum of available and occupied binding sites) was determined in the same cytosol fractions and nuclear extracts that served for the progesterone receptor assay. The total concentration of oestradiol receptors in cytosol and nucleus decreased significantly in both fetal and newborn oestradiol-treated animals (Table 2). In the newborn animals, this decrease was particularly marked in animals given 100 µg oestradiol.

DISCUSSION

Previous reports from this laboratory have shown that specific, high-affinity binding sites for oestradiol (Pasqualini et al. 1976; Sumida & Pasqualini, 1979a) and for progesterone and R5020 (Pasqualini & Nguyen, 1979a, b) can be found in the fetal guinea-pig uterus. When
the concentrations of progesterone and oestradiol receptors were studied in fetal uterine cytosol during the course of fetal development (Pasqualini et al. 1976; Pasqualini & Nguyen, 1979a), it was clear that the oestradiol receptor appeared much earlier (34–35 days of gestation) than did the progesterone receptor (45–49 days). From this observation, it was possible to suggest that oestradiol had a biological effect in a fetal tissue as has been observed in the uterus of immature and adult ovariectomized guinea-pigs (Falk & Bardin, 1970; Corvol, Falk, Freifeld & Bardin, 1972; Philibert & Raynaud, 1974), chick oviducts (Sherman, Corvol & O’Malley, 1970) and mouse and rat uteri (Feil, Glasser, Toft & O’Malley, 1972). Therefore, two parameters of oestrogenic response, the uterotrophic response and progesterone-receptor synthesis, after administration of 1 mg oestradiol to pregnant guinea-pigs for 1 or 3 days were tested in the fetal uterus as has been previously reported (Pasqualini & Nguyen, 1979a, b).

In the present study, the response of uterine wet weight and the levels of progesterone receptors were studied after only 24 h of oestradiol treatment and compared in the fetal and perinatal periods. In the fetus, uterine wet weight did not increase in response to oestradiol treatment within 24 h while an increase was evident in newborn animals. This inability of the fetal uterus to respond rapidly is in contrast with the observations in immature rat uteri (Anderson, Peck & Clark, 1973) where the early uterotrophic responses could be measured after only 3 h.

However, fetal levels of progesterone receptor responded rapidly to oestradiol treatment with a sevenfold increase after only 24 h reaching similar values to those obtained after the 3 consecutive days of treatment which were required to elicit a uterotrophic response (Pasqualini & Nguyen, 1979b). It was thus possible to separate two parameters of oestrogenic action, the uterotrophic response and the effect of oestradiol on the synthesis of a progesterone-receptor protein in the fetus.

The increase in progesterone receptor could not be due to contamination by progesterone-binding globulin (PBG) because progesterone binding in the fetal uterus does not have the same physicochemical characteristics as PBG in fetal plasma. The specific binding of progesterone in uterine cytosol is destroyed (90–95%) if this fraction is incubated for 1 h at 37 °C in the absence of the hormone while there is no effect on plasma protein binding. Moreover, R5020 binds specifically to receptor proteins in the cytosol fraction and not to proteins in fetal plasma. Furthermore, the 6–7S component in sucrose-density gradients is found only in the uterine cytosol preparation (Pasqualini, Sumida, Nguyen, Tardy & Gelly, 1980).

In newborn animals the basal levels of progesterone receptors were twice as high as in the fetal uterus and there was only a twofold increase in progesterone receptor 24 h after treatment. In the newborn animals it is possible that a longer period of repeated administration of oestradiol may be necessary for complete expression of progesterone-receptor stimulation. Nevertheless, it is interesting to note that the kinetics of the oestrogenic response are different in the perinatal and the fetal periods.

When the progesterone receptors increased in the uterus, oestradiol receptors decreased during the same period of 24 h. This observation in vivo confirms previous studies in vitro (Sumida & Pasqualini, 1979a). The phenomenon was unlike that observed in the immature rat uterus (Katzenellenbogen & Ferguson, 1975) in which oestrogen-receptor concentrations were increased 24 h after oestradiol administration. However, the observation was similar to that in a human breast cancer cell line (MCF-7) reported by Horwitz & McGuire (1978) who attributed the finding to receptor processing which is apparently necessary for progesterone-receptor stimulation in this cancer cell line. It has been suggested that the administration of oestrogen leads to a new steady-state level of oestrogen receptor. That this may be the case in the fetus is suggested by the observation that the fetal guinea-pig uterus has a much higher concentration of oestradiol receptor sites than the rat uterus (Sumida & Pasqualini, 1979a) and that it has a lower concentration of oestradiol in the plasma (Sumida...
& Pasqualini, 1979b). The receptor level decreases to a new steady-state level after administration of a very high concentration of oestradiol.

In conclusion, the results of the present work have shown that the fetal uterus can respond rapidly to treatment with oestradiol by increased concentrations of progesterone receptors and that this response also occurs, but to a lesser extent, in the perinatal period. This demonstrates that the hormonal effect of oestradiol can be expressed in the fetus, which can be used as a model for the study of uterotrophic responses distinct from the synthesis of progesterone receptors and for the study of regulation of oestradiol receptors.

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REFERENCES


