Glucose metabolism in perinatal gonads of the rabbit

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Summary. Metabolism of [U-14C]glucose was studied in the prenatal and neonatal rabbit ovary. Control tissues included the testis and female liver. No significant changes in glucose metabolism were observed in liver tissue. Mitosis and glucose oxidation were maximal in ovary and testis at 30 days post coitum and then declined dramatically by Day 8 after birth. Since mitosis is the primary physiological event in the gonad during the perinatal period these data suggest that glucose may be an important carbohydrate source for energy at this time.

Introduction

The energy requirements for most developing oocytes are provided by aerobic respiration and a source of pyruvate or oxaloacetate (Biggers, 1972; Eppig, 1976; Moor & Warnes, 1979). By contrast, there is a paucity of data on metabolic studies of the germ cells. Brinster & Harstad (1977) isolated germ cells from the genital ridge of fetal embryos of Day-15 pregnant mice and demonstrated that the utilization of pyruvate was 11 times greater than that of glucose. This ratio was similar in the newly ovulated oocyte and fertilized ovum, and became different at the blastocyst stage when both substrates were utilized equally well. Using the hamster model, Fajer (1983) found that there was a significant decrease (88%) in glucose metabolism between pre-meiotic and meiotic oocytes and concluded that this could be associated with the onset of meiosis.

In our continuing studies to examine the role of the pituitary in controlling ovarian function of the rabbit, the question arose as to whether the metabolic requirements of the developing germ cells were different. The rabbit model is unique since oogenesis does not begin until after birth and is completed within the first 3 weeks of life (Teplitz & Ohno, 1963; Peters, Levy & Crone, 1965). It is therefore possible to examine glucose metabolism before and during mitotic proliferation of gonocytes and during the progression of meiosis to prophase I. The present study has examined these changes.

Materials and Methods

New Zealand White rabbits were maintained under standard laboratory conditions and mated with a fertile buck. Dated neonatal rabbits on Days 1, 8 and 22 were obtained from local breeders. On Days 28 and 30 of pregnancy (Days 28 and 30 post coitum, term being 32 days) the does were killed by exsanguination and fetuses removed.

Ovaries, testes and pieces of liver were weighed and incubated in 1 ml Hank's balanced salt solution (Grand Island Biologicals, New York, U.S.A.) in a 5 ml Wheaton serum vial. Then 20 μl [U-14C]glucose (sp. act. 257-7 mCi/mmol: New England Nuclear, Boston, MA, U.S.A.) containing 2 μCi were mixed with the salt solution. Control vials contained tissue and no tracer. The vials were then capped with a soft rubber stopper from which was suspended a small tube with 200 μl 0.5 m-Protosol (New England Nuclear). Each serum vial contained 4 fetal gonads from Day 28 and 30 of pregnancy or neonatal gonads from animals within 24 h of birth, and 2 gonads from animals
on Day 8 or Day 22 after birth. Pieces of liver corresponding to gonadal weights were also used. The vials were incubated at 38°C in a Dubnoff metabolic shaker for 30 min. At the end of the incubation 1 ml 17% (w/v) phosphoric acid was injected through the rubber stopper and the Protosol was transferred to a scintillation vial. This was mixed with 200 µ1 absolute ethanol and 10 ml scintillation fluid. Radioactivity was measured in a Beckmann LS 5801 liquid scintillation counter with an efficiency of ~96%. Results were expressed as cpm $^{14}$CO$_2$ released per 10 mg wet weight of tissue.

Initial studies on enzyme kinetics showed that the metabolism of $[^{14}$C]glucose to $^{14}$CO$_2$ was linear to 1 h and then levelled off. For each litter at least 3 vials were used for control, zero time and 30 min. Thus each experiment represents values for a single litter, except for Days 8 and 22 after birth and for liver, for which more tissue was available.

Whenever possible tissues were fixed in Davidson’s fixative and processed for histology with haematoxylin and eosin staining.

Statistical analysis included t test, one-way analyses of variance and Duncan’s multiple range tests (Duncan, 1955).

Results and Discussion

The metabolism of [U-$^{14}$C]glucose to $^{14}$CO$_2$ by the various tissues is shown in Table 1. There was an 80% increase in glucose utilization by the fetal ovaries from Days 28 to Day 30 post coitum compared to 32% by the fetal testes at the same times. Glucose metabolism in the ovaries and testes was maximal on Day 30 post coitum being 151 and 348% higher respectively than the lowest level on Day 22 after birth. This high metabolic activity seems to be associated with the high mitotic activity of the germ cells (3-6 ± 0.7% of total oogonia) at this age as demonstrated by histological examination (unpublished data). Gondos & Byskov (1981) have also shown that the mitotic index of germ cells in the male rabbit fetus rises from 4-8 ± 1 on Day 28 to 7-3 ± 0.9 on Day 30 of pregnancy and is absent 5 days after birth. Meiotic cells are not present in prenatal or neonatal rabbit testes up to 3 weeks after birth (Gondos & Byskov, 1981). In contrast, mitotic activity of oogonia can be seen on the day of birth and averages 2-5% up to Day 5 after birth (YoungLai & Byskov, 1983). By Day 7–9 after birth more than 85% of germ cells are in meiosis with complete arrest of meiosis on Day 22 after birth (Peters et al., 1965; YoungLai & Byskov, 1983). In most mammals the period of mitosis precedes the onset of meiosis by several days (Mauleon, 1969; Gondos, 1978; Byskov & Grinsted, 1981). It is therefore possible that mitosis of germ cells may have a greater requirement for glucose.

Although the liver and testes were chosen as control tissues it is obvious from the data in Table 1 that each tissue has different metabolic patterns although testes and ovaries may be very similar.

<table>
<thead>
<tr>
<th>Age</th>
<th>Ovary</th>
<th>Testis</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 days of gestation</td>
<td>304±77 (3)c</td>
<td>386±35 (8)b</td>
<td>66±12 (10)b</td>
</tr>
<tr>
<td>30 days of gestation</td>
<td>546±61 (5)b</td>
<td>511±50 (9)a</td>
<td>103±9 (10)b</td>
</tr>
<tr>
<td>1 day after birth</td>
<td>427±31 (13)b</td>
<td>332±32 (10)b</td>
<td>285±42 (19)a</td>
</tr>
<tr>
<td>8 days after birth</td>
<td>278±22 (13)c</td>
<td>203±12 (16)c</td>
<td>146±10 (15)b</td>
</tr>
<tr>
<td>22 days after birth</td>
<td>217±25 (17)c</td>
<td>114±14 (10)b</td>
<td>198±41 (8)a</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for the number of determinations indicated in parentheses. Within tissues, values with identical superscripts were not significantly different from each other (Duncan’s multiple range test, $P > 0.05$). Differences between ovary and testis were significant on Day 1 ($P < 0.05$), Day 8 ($P < 0.01$) and Day 22 ($P < 0.005$) (t test).
The differences with the liver can be expected since each organ, e.g. heart, lungs and liver, matures at a specific time independent of others with respect to glycogen metabolism (Bhavnani, 1983). In addition, metabolism in the liver probably reflects changing needs of the fetus and neonate.

In the female rabbit meiosis does not begin at the time of sexual differentiation, a phenomenon referred to as delayed meiosis and attributed to different levels of meiosis-inhibiting and meiosis-preventing substances (Byskov & Grinsted, 1981). Also, the initiation of meiosis may occur in the presence of mitotic figures in oogonia leading to synchrony in the progression of meiosis (Mauleon, 1969). This may account for the higher utilization of glucose by Day 1 ovaries compared to testes. The contribution of the somatic cells to glucose metabolism has been neglected because at this early stage in development the greatest portion of the ovary is occupied by germ cells (Mauleon, 1969; Fajer, 1983).

The results of this study indicate that there is a peak in glucose utilization during ovarian development which corresponds to the age when mitosis is proceeding maximally. There was a 22% decrease in glucose utilization associated with the onset of meiosis on Day 1, a further 35% drop on Day 8 and 22% on Day 22 after birth. Although this trend is similar to the differences noted by Fajer (1983) for the hamster, he compared 3 time periods, the premeiotic being the 14th day post coitum and the meiotic Days 3 and 5 after birth, with the age difference being 5–7 days. In our study with a closer age difference of 2 days there was a 22% decrease in glucose metabolism associated with the onset of meiosis on Day 1 after birth compared to a 88% decline reported by Fajer (1983). However, in both studies it is evident that the progression of meiosis is associated with decreased utilization of glucose which may be a generalized phenomenon in mammalian germ cells.

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References


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