Effect of different methods of germinal cell destruction on rat testis

P. Dierickx and G. Verhoeven*

Laboratorium voor Experimentele Geneeskunde, Department of Developmental Biology, Faculty of Medicine, Katholieke Universiteit Leuven, Belgium

Summary. Germinal cell aplasia was induced in rats by heat sterilization, fetal irradiation or bilateral cryptorchidism. The influence of these treatments on the plasma concentration of LH and FSH, on the levels of sorbitol dehydrogenase and gamma-glutamyl transpeptidase (GTP) in the testis and on the weight of androgen target tissues was compared. All these methods damaged the germinal cells but also affected the other tubular cells and the interstitial compartment to a variable degree. Local heating affected plasma LH levels and GTP activity only minimally. Studies of GTP in cell-enriched fractions from the testes of rats with germinal cell aplasia indicated that this enzyme is not a Sertoli cell specific marker.

Introduction

Germinal cell aplasia (Sertoli cell-only syndrome) can be experimentally induced by a variety of methods: administration of busulphan during embryonic development (Heller & Jones, 1964), feeding a vitamin A-deficient diet (Krueger, Hodgen & Sherins, 1974; Rich & de Kretser, 1977), treatment with hydroxyurea (Rich & de Kretser, 1977), fetal irradiation (Fakunding, Tindall, Dedman, Mena & Means, 1976), surgical induction of bilateral cryptorchidism (Gupta, Rager, Zarzycki & Eichner, 1975) or heat sterilization (Collins & Lacy, 1969). In the present investigation we compared the influence of the last three methods on the activity of two testicular enzymes: sorbitol dehydrogenase, a marker of pachytene spermatocytes (Mills & Means, 1972), and gamma-glutamyl transpeptidase, supposedly a marker of Sertoli cells (Hodgen & Sherins, 1973), on the concentration of LH and FSH in plasma and on the androgenic status of rats.

Materials and Methods

Animals and treatment

Wistar R rats were obtained from a local breeding centre. Fetal irradiation (Beaumont, 1960) was performed on the 20th day of pregnancy as described by Fakunding et al. (1976). Experimental bilateral cryptorchidism was effected in 21-day-old rats as described by Gupta et al. (1975). Heat sterilization was obtained by exposing the scrotal region of 76-day-old rats to 43°C for 30 min (Collins & Lacy, 1969). At 90 days of age all animals were killed by decapitation and exsanguinated. Testes, kidneys, ventral prostate glands, seminal vesicles and hypophysis were removed and weighed.

* Present address: Rega Instituut, Minderbroedersstraat 10, 3000 Leuven, Belgium.
Enzyme assays

Testes were frozen in liquid nitrogen and stored at -20°C before assay of sorbitol dehydrogenase (EC 1.1.1.14) activity. The tunica albuginea was removed and the testicular tissue was weighed, minced and homogenized in 9 volumes of Tris-EDTA buffer (20 mM-Tris chloride and 1.5 mM-EDTA), pH 6.8, for three 10-sec periods (Ultraturrax model TP-10N). The temperature was carefully maintained below 4°C. The homogenates were centrifuged for 45 min at 150 000 g and sorbitol dehydrogenase activity was measured in the high-speed supernatant as described by Mills & Means (1972). Gamma-glutamyl transpeptidase (EC 2.3.2.2) activity was determined by a modification of the method of Szasz (1969). A high-speed supernatant was prepared as described above but homogenization was performed in 0.185 M-Tris chloride, pH 8.25, containing 154 mM-NaCl and 1% Triton X-100. The reaction mixture contained 80 mM-glycylglycine and 4 mM gamma-L-glutamyl-p-nitroanilide in 1.5 ml of this homogenization buffer. An aliquot (0.1 ml) of the high-speed supernatant, appropriately diluted, was added and the increase in absorbance at 405 nm was recorded for 5 min at 24°C. Enzyme activities are expressed in milliunits. One unit (U) is defined as the amount of enzyme that catalyses the formation of 1 μmol product/min under the assay conditions.

Hormone assays

Plasma gonadotrophins were measured by double-antibody radioimmunoassay in the laboratory of Dr Carl Denef (Department of Pharmacology, Leuven) using the kits distributed by the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Distribution Program, Bethesda, Maryland, U.S.A. Hormones for radiiodination were rat LH-Iγ and rat FSH-Iγ. Iodination was with 125I (Amersham). The antisera were anti-rat-LH-S3 and anti-rat-FSH-S3. The anti-rabbit γ-globulin was produced in goats at this university. Samples were assayed in duplicate. Calculations were done on a logit-log transformed standard curve. NIAMDD-Rat-FSH-RP1 and NIAMDD-rat-LH-RP1 were used as reference preparations. Sensitivity was 15.3 ± 3.0 and 2.9 ± 0.6 ng/ml (mean ± s.e.m.; n = 10) for FSH and LH assays respectively. Mean precision (λ) at the 50% inhibition points was 0.035 ± 0.006 for FSH and 0.051 ± 0.008 for LH (mean ± s.e.m.; n = 35). All samples were measured within the same assay. Within-assay variance, as estimated by the coefficient of variation (5 assays each of 5 duplicates) was 4.15% within a range of 100–200 ng FSH and 8.31% within a range of 10–20 ng LH (Denef, Hautekeete & Dewals, 1978).

Separation of testicular cells

For some experiments testes were divided into three portions for enrichment of interstitial cells, peritubular cells and Sertoli cells. Separation by treatments with collagenase and pancreatin was performed by a modification of the method of Welsh & Wiebe (1975), as described by Verhoeven, Dierickx & De Moor (1979). This procedure produces relatively pure (~80%) fractions of Sertoli cells and Leydig cells, as judged microscopically, histochemically, by the ability to secrete testosterone and by aromatizing ability. The peritubular fraction, however, is contaminated by Leydig cells and Sertoli cells (Verhoeven, 1979). After homogenization of each fraction, a 10 000 g high-speed supernatant was prepared in buffer containing 1% Triton X-100 and assayed for gamma-glutamyl transpeptidase activity.

Results

The three methods of sterilization resulted in a comparable fall in testicular weight as can be judged from Table 1. There was a slight but statistically significant reduction of seminal vesicle weight in the animals irradiated as fetuses but no other accessory sex organ indication of an
altered androgen balance. The activity of sorbitol dehydrogenase was reduced in all 3 experimental groups (Table 1), but less so after heat sterilization. Gamma-glutamyl transpeptidase activity increased to a variable degree when expressed per g testis, but the total testicular content of this enzyme was reduced, albeit less markedly than was sorbitol dehydrogenase. There was a significant increase in plasma FSH concentrations in all the experimental groups (Table 1) and of LH values in the irradiated and cryptorchid animals. The variation of FSH and LH concentrations was considerably smaller in the irradiated animals.

<table>
<thead>
<tr>
<th>Table 1. Influence of germinal cell destruction on various characteristics of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat sterilization</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Organ weights (mg/100 g body weight)</td>
</tr>
<tr>
<td>Testes</td>
</tr>
<tr>
<td>Ventral prostate</td>
</tr>
<tr>
<td>Seminal vesicles</td>
</tr>
<tr>
<td>Adrenals</td>
</tr>
<tr>
<td>Hypophysis</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (mU/g)</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (mU/2 testes)</td>
</tr>
<tr>
<td>Gamma-glutamyl transpeptidase (mg/ml)</td>
</tr>
<tr>
<td>Gamma-glutamyl transpeptidase (mg/2 testes)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for the animals indicated in the columns or in parentheses when different. Values significantly different (Student’s t test) from their equivalent control, * P < 0.05; † P < 0.01; ‡ P < 0.001.

Separation experiments

In 3 experiments with normal testes 79 ± 10% of the gamma-glutamyl transpeptidase activity was found in the tubular fraction and 21 ± 10% in the interstitial cell fraction, in spite of the fact that the fractions prepared from the testes of normal animals are always contaminated with spermatogenic cells. In three experiments with heat-sterilized rats 18 ± 6% (s.d.) of total testicular gamma-glutamyl transpeptidase activity was found in the interstitial cells, 38 ± 3% in the peritubular cells, and 44 ± 6% in the Sertoli cells. The specific activity did not differ markedly between the three fractions, being 6-7 ± 2-7, 7-4 ± 0-9 and 6-4 ± 1-3 U/g protein respectively. In a single experiment with irradiated rats the distribution of this enzyme in these cell fractions was 20% in interstitial cells (specific activity 2-83), 36% in peritubular cells (specific activity 4-93) and 44% in Sertoli cells (specific activity 7-11).

Discussion

The marked reduction in testicular weight and in SDH activity shows that heat sterilization, fetal irradiation and experimental cryptorchidism are effective methods to induce germinal cell aplasia. The influence of these treatments, however, is not limited to the germinal epithelium. Although the androgenic status was generally not altered, the plasma concentration of LH was...
clearly raised. These findings do not agree with the data of Krueger et al. (1974) who found reduced androgen and LH values in chronic vitamin A-deficient rats. They are in accordance, however, with the data of Gupta et al. (1975) for cryptorchid rats, of Rich & de Kretser (1977) for fetal-irradiated, hydroxyurea-treated or vitamin A-deficient rats, and of Aafjes, Vreeburg & Schenck (1978) for heat-sterilized rats. This disturbance of the testicular–hypophysial–hypothalamic axis suggests that either the number of Leydig cells or their response to LH is affected directly or indirectly by these sterilization procedures.

In our study, the plasma concentrations of FSH were raised to a comparable degree by the three treatments, but although FSH can be suppressed by androgens in rats with germinal cell aplasia (Aafjes et al., 1978) it is likely that some other factor derived from the germinal cells or the Sertoli cells is also involved in the control of FSH secretion (Krueger et al., 1974; Rich & de Kretser, 1977; de Jong & Sharpe, 1977; Chowdhury, Steinberger & Steinberger, 1978; Main, Davies & Setchell, 1978). Gamma-glutamyl transpeptidase has been proposed as a potential biochemical marker of Sertoli cells (Hodgén & Sherins, 1973) and has been included as such in this study. The increase in the specific activity of this enzyme after sterilization accords with the observations of Krueger et al. (1974) and indicates that gamma-glutamyl transpeptidase is localized mainly or exclusively outside the germinal epithelium. The decrease in the total testicular content of gamma-glutamyl transpeptidase, however, suggests either that germinal cells contain some activity or that Sertoli cells or other non-germinal cells (interstitial cells or peritubular cells) are also damaged by the sterilization procedures. The latter hypothesis is supported by morphological studies showing that irradiated rats have fewer Sertoli cells (de Jong & Sharpe, 1977) and by the observation that the production of androgen-binding protein (ABP), a protein secreted by Sertoli cells, is also impaired in cryptorchid rats (Hagenas & Ritzen, 1976), in fetal-irradiated rats, and in animals treated with hydroxyurea or chronic feeding of a vitamin A-deficient diet (Rich & de Kretser, 1977). Abnormal Sertoli cell function has also been deduced from the disturbances observed in K⁺ concentrations in tubular fluid from Busulphan-treated and fetal-irradiated rats (Levine & Marsh, 1975; Setchell et al., 1978). Histological analysis might reveal whether interstitial and peritubular cells are also damaged.

Our studies on the distribution of GTP activity in rats with experimentally induced germinal cell aplasia indicate that in these animals GTP may not be an ideal marker enzyme for Sertoli cells. In fact, the specific activity of GTP in the two purer fractions (interstitial cells and Sertoli cells) did not differ markedly despite the fact that 80% of the enzyme activity is localized in the peritubular or tubular cells. Although cell losses or cell damage during the separation procedure and cross contamination of the different fractions may in part be responsible for this observation, further studies on the exact localization of GTP in the testis seem to be required.

G.V. is “aangesteld navorser” of the Nationaal Fonds voor Wetenschappelijk Onderzoek of Belgium. We thank Dr C. Denef for the measurements of LH and FSH, the NIH for the hormone assay materials; and Mrs Chris Hekkert-Meulemans for expert technical assistance.

References


Denef, D., Hautekeete, E. & Dewals, R. (1978) Mono-


Received 11 June 1979