Photoperiod-induced changes in the testicular metabolism of [4-\(^{14}\)C]17\(\alpha\)-hydroxyprogesterone in the bank vole (Clethrionomys glareolus)

T. Teräväinen and K. M. Tähkä*

Department of Medical Biology, University of Helsinki, Siltavuorenpuenger 20 A, SF-00170 and *Division of Physiology, Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100, Finland

Summary. Minces of the testes of bank voles, born and reared in a long (18L:6D) photoperiod until weaning (18–22 days of age) and subjected thereafter to a short (6L:18D, Group S) or a long (18L:6D, Group L) photoperiod for 6–9 weeks, were incubated with [4-\(^{14}\)C]17\(\alpha\)-hydroxyprogesterone in the presence of cofactors (NADP/ NADPH, 1–3 mmol/l) for 1 h at 37°C. The radioactive metabolites were characterized and identified by thin-layer chromatography with derivative formation and chromatography to constant specific activity and isotope ratio. In Group L virtually all of the substrate was utilized and it was readily converted to androgens (48% of the radioactivity recovered) such as androstenedione and testosterone. The only pregnane metabolite identified was 17\(\alpha\)-hydroxy,20\(\alpha\)-dihydroxyprogesterone (43.3%). In Group S there was a decreased production of 17\(\alpha\)-hydroxy,20\(\alpha\)-dihydroprogesterone and androgens (25.4% and 10.4% respectively) and a substantial portion of the substrate was not metabolized (38.8%). The main androgen metabolites identified, androst-4-en-3\(\beta\),17\(\beta\)-diol and 5\(\alpha\)-androstan-3,17-dione are hormonally quite inert steroids. No androstenedione or testosterone was found. The results indicate that exposure to short photoperiod induces a decrease in the testicular C\(_{17}-C_{20}\) lyase and 20\(\alpha\)-hydroxysteroid dehydrogenase.

Introduction

There is considerable age-specific and interspecies variation in the enzymic pathways used for testicular androgen synthesis in mammals (Mizutami, Tsujimura, Akashi & Matsumoto, 1977; Preslock & Steinberger, 1977; Ewing et al., 1979; Preslock, 1980; Terada, Sato & Matsumoto, 1980). The existence of such biosynthetic variability does not necessarily mean that no general regulatory principles exist in the control of androgen synthesis in mammals. The acute effects of LH on testicular testosterone synthesis are exerted mainly on the enzymes catalysing the side-chain cleavage of cholesterol (Catt et al., 1980).

In many rodents the production of hormonally active androgens is reduced during the prepubertal period by a shift from the synthesis of Δ\(^{4}\)-3-keto C\(_{19}\)-steroids to that of hormonally inert 5\(\alpha\)- and 5\(\beta\)-reduced androgens (Moger & Armstrong, 1974; Terada et al., 1980). Some studies of rats suggest that there are regulatory systems operating at the testicular level controlling testosterone synthesis through the action of steroids and peptides produced locally in the Leydig cells or the seminiferous epithelium (Nozu, Deheja, Zawistowich, Catt & Dufau, 1981; Adashi & Hsueh, 1982; Sharpe, Fraser & Cooper, 1982). The key steroidogenic enzymes controlled by these intratesticular regulators appear to be 17\(\alpha\)-hydroxylase and C\(_{17}-C_{20}\) lyase.
In a series of studies investigating the control of gonadal function in seasonally breeding mammals, we have studied the effects of photoperiod on gametogenesis and steroid synthesis in the testis of the bank vole (Clethrionomys glareolus) (Tähkä, 1978; Tähkä, Teräväinen & Wallgren, 1982, 1983b; Tähkä, Ruokonen, Wallgren & Teräväinen, 1983a). Photoperiod affected testicular 17α-hydroxylase activity, i.e. the same enzyme modulated by intratesticularly produced oestrogen or by the paracrine regulatory substances presumed to exist in the rat testis (Purvis, Cusan & Hansson, 1981; Sharpe et al., 1982). It therefore seemed possible that gonadal maturation and regression in seasonally breeding species is not only accompanied by changes in hypothalamo-hypophysial function, but also by changes in the local regulatory mechanisms in the testis. The present study was undertaken to determine whether the activity of C17-20 lyase (EC 4.1.99) as well as that of 17α-hydroxylase (EC 1.14.99.9) in vitro is modulated by photoperiod, a fact that could not be established in our previous studies (Tähkä et al., 1982, 1983b).

Materials and Methods

Animals. Juvenile male bank voles, from our own laboratory stock, were subjected after weaning (18–22 days of age) to a long (Group L, 18L:6D, lights on 06:00–24:00 h) or short (Group S, 6L:18D, lights on 08:00–14:00 h) photoperiod for 6–9 weeks. Each experimental group consisted of 30 voles. The experimental conditions (caging, feeding, lighting) have been described in more detail elsewhere (Tähkä, 1978). The animals were killed by decapitation and the testes were excised. The left testis was weighed and stored in liquid nitrogen. The incubations were performed within 3 h of death of the animals.

Chemicals. [4-14C]17α-Hydroxyprogesterone (sp. act. 61 mCi/mmol), [1,2,6,7,(n)-3H]androstenedione (sp. act. 87 Ci/mmol), [1,2,6,7,(n)-3H]17α-hydroxyprogesterone (sp. act. 60 Ci/mmol) and [1,2,6,7,(n)-3H]testosterone (sp. act. 93·9 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, U.K.) or from New England Nuclear (Boston, U.S.A.). The purity of the radioactive steroids was checked by thin-layer chromatography (t.l.c.) before use. Non-radioactive steroids and reagents were obtained and their purity assessed as previously described (Tähkä et al., 1983b).

Incubations. Testicular minces (~100 mg) consisting of pooled material from the testes of 5–6 voles were incubated in the presence of exogenous cofactors (NADP/NADPH 1·3 mmol/l) and [4-14C]17α-hydroxyprogesterone (0·113 μCi dissolved in 50 μl propyleneglycol/ethanol, 1:1, v/v) in 3 ml 0·05 M-Tris buffer at 37°C for 1 h. The incubations were performed under continuous agitation and 95% O2/5% CO2 flow and were terminated by the addition of hot acetone (1·5 ml) to the incubation medium. Appropriate controls with acetone-denatured minces and identical concentrations of cofactors and substrate were also carried out. In each experimental group, 5 incubations were performed.

Extraction and characterization of the steroid metabolites. The extraction procedure as well as the chromatographic methods used for the identification of steroids (bidimensional t.l.c., derivative formation, determination of constant specific activity or constant isotope ratio) have been described in detail earlier (Kaartinen, Laukkanen & Saure, 1971; Teräväinen & Saure, 1976; Antila, 1977; Tähkä et al., 1982). The following solvent systems were used: (1) chloroform : acetone, 85:15 (v/v); (2) chloroform : ether, 3:1 (v/v); (3) cyclohexane : ethyl acetate : ethanol, 9:9:1 (by vol.); (4) cyclohexane : ethyl acetate, 1:1 (v/v); (5a) chloroform : ethanol, 19:1 (v/v); (5b) chloroform : ethanol, 9:1 (v/v); (6) dichloromethane : acetone, 5:1 (v/v); and (7) benzene : ethanol, 19:1 (v/v).
Results

Testicular weight

As previously found (Tähkä, 1978) there was a striking difference in the mean (± s.d.) testicular weight of the experimental groups (367.6 ± 50.1 mg in Group L and 10.8 ± 4.1 mg in Group S).

17α-Hydroxyprogesterone metabolism

The mean (± s.d., n = 5) recovery of radioactivity extracted in ether (after partition against 1 N-NaOH and subsequent washing with NaHCO₃) was 85.7 ± 3.9% in Group L and 92.3 ± 3.8% in Group S, indicating that most of the metabolites formed were neutral steroids. No attempt was made to study the possible photoperiod-induced changes in the in-vitro production of conjugated and phenolic steroids. After the first t.l.c. (System 1) the corresponding fractions within each experimental group were pooled and characterized further by bidimensional chromatography with appropriate carrier steroids. The final identification of radioactive steroids, with the exception of 17α-hydroxyprogesterone (fraction 6, Group L) and 5α-androstanedione (fraction 8, Group S), was made by chromatography to constant isotope ratio or constant specific activity (Tables 1 and 2). The identity of these two compounds remained uncertain, since their characterization was based only on isopolarity with authentic unlabelled steroids in bidimensional t.l.c.

Table 1. Determination of constant specific activity of 17α-hydroxy,20α-dihydroprogesterone in Fraction 3

<table>
<thead>
<tr>
<th>Chromatography step</th>
<th>Solvent system*</th>
<th>Group L (c.p.m./µg)</th>
<th>Group S (c.p.m./µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td>375</td>
<td>330</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>345</td>
<td>307</td>
</tr>
<tr>
<td>2</td>
<td>5a</td>
<td>370</td>
<td>301</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>342</td>
<td>313</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td></td>
<td>352 ± 15</td>
<td>307 ± 6</td>
</tr>
</tbody>
</table>

* See text.

Table 2. Determination of constant isotope ratio of testosterone, androstenedione, androstenediol and 17α-hydroxyprogesterone

<table>
<thead>
<tr>
<th>Chromatography step</th>
<th>Solvent system*</th>
<th>Testosterone (Group L, Fraction 5)</th>
<th>Androstenedione (Group L, Fraction 7)</th>
<th>Androstenediol† (Group S, Fraction 4)</th>
<th>Androstenediol† (Group L, Fraction 4)</th>
<th>17α-Hydroxyprogesterone (Group S, Fraction 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td>650</td>
<td>548</td>
<td>660</td>
<td>515</td>
<td>1070</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>540</td>
<td>514</td>
<td>620</td>
<td>470</td>
<td>960</td>
</tr>
<tr>
<td>2</td>
<td>5a</td>
<td>588</td>
<td>501</td>
<td>651</td>
<td>470</td>
<td>890</td>
</tr>
<tr>
<td>3</td>
<td>6, 7†</td>
<td>547</td>
<td>508</td>
<td>593</td>
<td>442</td>
<td>903</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td></td>
<td>558 ± 26</td>
<td>508 ± 7</td>
<td>621 ± 29</td>
<td>460 ± 16</td>
<td>917 ± 37</td>
</tr>
</tbody>
</table>

* See text.
† Androstenediol was oxidized with CrO₃ to androstenedione before determination of constant isotope ratio.
‡ Used only when identifying Fraction 7, Group S.
Table 3. Percentage conversion of [4-¹⁴C]l 7α-hydroxyprogesterone to metabolites after incubation for 1 h in the presence of exogenous cofactors (NADP/NADPH: 1·3 mmol/l).

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Group L (18L:6D)</th>
<th>Group S (6L:18D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-Hydroxyprogesterone</td>
<td>2·7 ± 0·6</td>
<td>38·8 ± 9·0</td>
</tr>
<tr>
<td>17α-Hydroxy, 20α-dihydroprogesterone</td>
<td>43·3 ± 5·0</td>
<td>25·4 ± 4·0</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>12·5 ± 2·8</td>
<td>—</td>
</tr>
<tr>
<td>Testosterone</td>
<td>24·7 ± 1·4</td>
<td>—</td>
</tr>
<tr>
<td>4-Androstene-3β,17β-diol</td>
<td>10·8 ± 2·0</td>
<td>3·4 ± 0·5</td>
</tr>
<tr>
<td>5α-Androstanedione</td>
<td>7·0 ± 6·0</td>
<td>—</td>
</tr>
<tr>
<td>Unidentified compounds</td>
<td>6·0 ± 1·0</td>
<td>25·4 ± 6·9</td>
</tr>
</tbody>
</table>

Values are mean ± s.d for 5 incubations.

The percentage conversion of [4-¹⁴C]l 7α-hydroxyprogesterone to metabolites is shown in Table 3. In the spermatogenically active testes of Group L voles almost all of the substrate was utilized and it was converted readily to androgens (48% of the radioactivity recovered), the bulk of which was androstenedione and testosterone. Group L testes produced also substantial amounts of 17α-hydroxy, 20α-dihydroprogesterone.

In the testes of Group S voles the substrate was not utilized as effectively (38% of unmetabolized substrate) and there was a reduced production of 17α-hydroxy, 20α-dihydroprogesterone and androgens (10·4%) (androstenediol and 5α-androstanedione). A substantially larger amount of unidentified metabolites were produced in Group S than in Group L incubations.

Discussion

Our present study suggests that light deprivation in the bank vole induces a decrease in testicular C₁₇-₂₀ lyase and 20α-hydroxysteroid dehydrogenase activities in vitro. To a large extent these changes are brought about by a photoperiod-induced decrease in pituitary LH, FSH and prolactin secretion mediated by the pineal gland (Craven & Clarke, 1982; Yellon & Goldman, 1984). At the testicular level the reduced 20α-hydroxysteroid dehydrogenase activity is probably due to the decrease in the relative volume of tubular tissue in relation to interstitial tissue in the regressed testis, since this enzyme is considered to reside mostly in the tubular compartment (van der Molen & Rommerts, 1981). Pudney & Lacy (1977) studied seasonal changes in the in-vitro metabolism of radiolabelled pregnenolone and progesterone in interstitial and tubular tissue isolated from the testes of the grey squirrel (Sciurus carolinensis) and found a decrease in the activity of 17α-hydroxylase and C₁₇-₂₀ lyase in both testicular compartments and an increase in the 20α-hydroxysteroid dehydrogenase activity in the interstitium of the regressed testis.

Our findings are not necessarily in disagreement with these results, since in our studies heterogeneous tissue was used. These studies are also not necessarily comparable, since the material of Pudney & Lacy (1977) consisted of animals that had attained sexual maturity before seasonal regression. It has been proposed that 20α-hydroxysteroid dehydrogenase could regulate androgen synthesis by competing with 17α-hydroxylase and C₁₇-₂₀ lyase for common substrates as well as by producing 20α-dihydropregnenolone, 20α-dihydroprogesterone and 17α-hydroxy, 20α-dihydroprogesterone which are known inhibitors of 17α-hydroxylase and C₁₇-₂₀ lyase in the human and rat testes (Shikita & Tamaoki, 1965: Fan, Oshima, Troen & Troen, 1974; Hosaka, Oshima & Troen, 1980). Indeed, 20α-hydroxysteroid dehydrogenase seems to have a regulatory role in the control of...
progesterone synthesis in the rat ovary during the luteinization of granulosa cells (Jones & Hsueh, 1981; Sharpe, 1982). However, there is no conclusive evidence for the existence of such a regulatory system in the testis of seasonally breeding mammals (de Bruijn & van der Molen, 1974; Pudney & Lacy, 1977).

The inhibition of C17-20 lyase activities in Group S voles is most probably due to functional changes in the interstitial compartment, since this enzyme resides almost entirely in the smooth endoplasmic reticulum of Leydig cells (van der Molen & Rommerts, 1981). In our previous in-vitro studies (Tähkä et al., 1982, 1983b), short photoperiods also seemed to induce a marked decrease in the activity of 17α-hydroxylase whereas no marked changes in the activity of 3β- and 17β-hydroxysteroid dehydrogenase were noted. However, the experimental conditions prevailing in a static in-vitro incubation system such as used in this study, give rise to many experimental artefacts (e.g. lack of intact circulation, differential substrate penetration, cofactor availability). Our in-vivo studies (Tähkä et al., 1983a), however, seem to support the regulatory role of 17α-hydroxylase and C17-20 lyase. The key role of these enzymes in the control of testicular androgen synthesis during gonadal maturation and seasonal regression may be a more general phenomenon amongst seasonally breeding mammals (Lincoln, 1981; Bartke, Klemcke, Amador & Van Sickle, 1982; Bedrak, Rosentrauch, Kafka & Friendlander, 1983). The decrease in 17α-hydroxylase activities and C17-20 lyase activities in the regressed testes could be due to a reduced level of cytochrome P-450 which is known to be essential for the function of these enzymes or to a selective decrease in subpopulations of Leydig cells rich in C17-20 lyase activity (Rosentrauch, Bedrak & Friendlander, 1978; Payne, O'Shaughnessy, Chase, Dixon & Christensen, 1982; Bedrak et al., 1983; Menard & Purvis, 1973; Nozu et al., 1982). Evidence has also accumulated that in addition to LH other substances such as an intratesticularly produced LHRH-like peptide, arginine vasotocin (a potential pineal antagonadotropic factor), as well as glucocorticoids and oestrogen, modulate, through a receptor-mediated process, 17α-hydroxylase and C17-20 lyase activities in the Leydig cell (Purvis, Clausen & Hansson, 1978; Nozu et al., 1981; Adashi & Hsueh, 1982; Welsh, Bambino & Hsueh, 1982; Sharpe et al., 1982; Hsueh, Bambino, Zhuang, Welsh & Ling, 1983). The regulatory interactions taking place at the testicular level therefore seem to be far more complicated than was initially envisaged.

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References


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