Incomplete androgen insensitivity:
asymmetry in morphology
and steroid profile and metabolism of the gonads.
An analysis of a case

Bengt Fredricsson¹, Kjell Carlström¹,
Berndt Kjessler⁵, Jan Lindstedt⁶, Leif Plöen⁴, Martin Ritzén³
and Bartolome de la Torre²

Department of Obstetrics and Gynaecology¹, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden
Reproductive Endocrinology Research Unit², and
Paediatric Endocrinology Unit, Department of Paediatrics³ at Karolinska and S:t Göran’s Hospital, Stockholm,
Department of Anatomy and Histology⁴,
Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden and
Department of Obstetrics and Gynaecology, University of Linköping⁵ and Betaniahemmet Hospital⁶, Stockholm

Abstract. A patient with clinical manifestations of the incomplete androgen insensitivity syndrome was studied with respect to peripheral blood levels of steroids and steroid sulphates before, during, and after gonadectomy. Steroid and steroid sulphate concentrations were also analyzed in spermatic venous blood and gonadal tissue collected during surgery. The metabolic capacity of gonadal tissue was also studied in vitro using progesterone, dehydroepiandrosterone sulphate and oestrone sulphate as substrates.

Profound differences between the two gonads were noted with respect to both steroid content and release into pampiniform veins and to in vitro conversion of progesterone and oestrone sulphate. Histological examination revealed the presence of seminiferous tubules with carcinoma in situ in both gonads.

It is suggested that the differences between the gonads may be due to an autonomous steroid production in the right gonad in spite of adequate or even elevated gonadotrophic stimulation resulting in a steroidogenic situation resembling the complete androgen insensitivity syndrome, while the conditions found in the left gonad more resembles the incomplete form of the disease.

The syndrome of testicular feminization is considered to be due to androgen receptor deficiency. Therefore, gonadotrophic stimulation and steroidogenesis would be expected to be enhanced. However, sometimes selected enzymatic steps involved in testosterone production may be deficient (Bell 1975). A case is here presented with fundamental differences between the two gonads with respect to both morphology, steroid content and steroid metabolism.

Case Description and Methods

A 35-year old Arabian woman sought advice because of primary amenorrhoea and infertility. She had experienced pubertal development at the age of 13 to 14 years and had always been healthy. On examination ordinary female sex characteristics were apparent, breasts were well developed and pubic hair growth was present. No uterus could be found and the vagina ended blindly. In the right groin a plum-sized tumour was found, later identified as the gonad. The left gonad was not accessible to examination. Chromosome analysis of short-term cultured lymphocytes revealed the karyotype 46,XY (Q- and G-banding). The clinical diagnosis of incomplete testicular feminization was based on these findings together with the histologic picture of the gonads, in accordance with the definition given by Rosenfield et al. (1971).
Bilateral gonadectomy was performed by laparotomy. The right gonad was located in the inguinal canal and measured 4 cm in diameter. It was solid and the cut surface was yellow and slightly nodulated. The left gonad was situated at the pelvic inlet. It measured 1.5 x 5 cm and was rather soft in consistency. The cut surface was greyish and appeared to contain more connective tissue than the right. There was no well-defined capsule. No adenomatous structures were observed.

The histological picture (Figs. 1–4) of the two gonads was essentially the same with the exception that connective tissue took up a greater part of the left gonad than of the right (Fig. 2). In both gonads seminiferous tubules of prepubertal character were present. Some tubules showed heavy degeneration or even complete hyalinization. Several other tubules of both gonads contained seminiferous epithelium which had changed into carcinoma in situ (Fig. 3). Leydig cells did not show any special features but were in both gonads often arranged in smaller or larger nodules (Fig. 4).

Before and after surgery and prior to institution of substitution therapy, peripheral levels of gonadotrophins, testosterone (T), 4-androsterone-3,17-dione (A-4), dehydroepiandrosterone (DHA) and its sulphate (DHAS), 17α-hydroxyprogesterone (17OHP), unjugated (E₁) and total (tE₁) oestrone were analyzed by radioimmunological techniques as described previously (Brody et al. 1982, 1983a,b; Fredricsson & Carlström 1979). Sex hormone binding globulin (SHBG) was analyzed by an immunoradiometric procedure using a commercial kit obtained from Farmos Diagnostica OY, Turku, Finland.

During the operation peripheral and pampiniform venous blood was drawn before extirpation of the gonads. Steroid content (T, A-4, DHA, 5-pregnenolone (P-5), 5α-dihydrotestosterone (DHT) and their sulphates (S)) was assayed in these samples and in tissue from both gonads as well employing methods adapted for such purposes (de la Torre et al. 1982).

The metabolism of tritiated progesterone, oestrone sulphate and DHAS in vitro was studied using total homogenates from both gonads. The study of the conversion pattern for progesterone included incubation of the steroid with tissue homogenate in phosphate (Buchner) buffer in air at 37°C and in the presence of excess NADPH, extraction with chloroform and separation of the metabolites by thin layer chromatography before and after acetylation (Fredricsson & Carlström 1979). Oestrone sulphatase and 17β-hydroxysteroid oxidoreductase activities were studied by a similar pro-

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Fig. 1.
Overview of section from the right gonad. Dominance of tubules (T) of prepubertal appearance. A few Leydig cells (arrows) can be seen. Stieve’s fixative, haematoxylin-eosin stain. × 160.
Overview of section from the left gonad. Apart from tubules (T) of prepubertal character, a small nest of Leydig cells (arrows) is seen. Stieve's fixative, haematoxylin-eosin stain, × 160.

Results

Peripheral hormone levels before and after surgery are given in Table 1. All hormone values before surgery were well within our normal reference limits for males except LH which was slightly elevated. After surgery, T, 17OHP and gonadotrophins changed into castrate or postmenopausal levels. No consistent changes after surgery were

<table>
<thead>
<tr>
<th></th>
<th>FSH U/l</th>
<th>LH U/l</th>
<th>T nM</th>
<th>A-4 nM</th>
<th>DHA nM</th>
<th>DHAS nM</th>
<th>17OHP nM</th>
<th>E1 pM</th>
<th>tE1 nM</th>
<th>SHBG nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day before surgery</td>
<td>2</td>
<td>22</td>
<td>30.4</td>
<td>7.3</td>
<td>22.8</td>
<td>3030</td>
<td>3.1</td>
<td>360</td>
<td>2.30</td>
<td>99</td>
</tr>
<tr>
<td>4 days after surgery</td>
<td>41</td>
<td>40</td>
<td>0.9</td>
<td>8.2</td>
<td>9.6</td>
<td>3393</td>
<td>0.9</td>
<td>*</td>
<td>1.43</td>
<td>96</td>
</tr>
<tr>
<td>10 days after surgery</td>
<td>44</td>
<td>51</td>
<td>1.2</td>
<td>5.0</td>
<td>14.0</td>
<td>6929</td>
<td>*</td>
<td>430</td>
<td>0.54</td>
<td>*</td>
</tr>
</tbody>
</table>

* Not determined due to lack of serum. ¹ Human pituitary FSH 68/39. ² Human pituitary LH or ICSH 68/40.
Seminiferous tubule of the right gonad showing cytological changes corresponding to carcinoma in situ (arrows).
Stieve's fixative, haematoxylin-eosin stain, × 400.

noted with regard to A-4 and E₁ whereas tE₁ and DHA levels became lower and DHAS attained a higher level after the operation. Serum SHBG was around the upper limit for normal females and did not change after gonadectomy.

Steroid and steroid sulphate levels in peripheral and spermatic venous blood during surgery and in gonadal tissue are given in Table 2. The levels of the unconjugated steroids studied were considerably higher in the right spermatic vein than in the

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Peripheral serum</th>
<th>Vena sperm. right</th>
<th>Vena sperm. left</th>
<th>Tissue right</th>
<th>Tissue left</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5</td>
<td>9.6 (5.8–7.7)</td>
<td>110</td>
<td>41.1</td>
<td>7294</td>
<td>430 (2100–3300)</td>
</tr>
<tr>
<td>DHA</td>
<td>35.3 (14–18)</td>
<td>507</td>
<td>93</td>
<td>1163</td>
<td>152 (670–1500)</td>
</tr>
<tr>
<td>A-4</td>
<td>9.3 (2.7–3.5)</td>
<td>445</td>
<td>41.7</td>
<td>769</td>
<td>56 (160–300)</td>
</tr>
<tr>
<td>T</td>
<td>13.4 (15–18)</td>
<td>1576</td>
<td>148</td>
<td>2562</td>
<td>76 (2200–3400)</td>
</tr>
<tr>
<td>DHT</td>
<td>0.9 (1.9–2.3)</td>
<td>69</td>
<td>15.8</td>
<td>1410</td>
<td>148 (80–120)</td>
</tr>
<tr>
<td>P5-S</td>
<td>97.6 (110–140)</td>
<td>187</td>
<td>161</td>
<td>3819</td>
<td>547 (1500–3300)</td>
</tr>
<tr>
<td>DHAS</td>
<td>865 (2700–3800)</td>
<td>817</td>
<td>614</td>
<td>7111</td>
<td>805 (1400–2400)</td>
</tr>
<tr>
<td>T-S</td>
<td>11.4 (9.0–14)</td>
<td>80.0</td>
<td>16.3</td>
<td>739</td>
<td>108 (230–480)</td>
</tr>
<tr>
<td>DHT-S</td>
<td>32.0 (70–86)</td>
<td>52.8</td>
<td>41.6</td>
<td>217</td>
<td>58.6 (100–150)</td>
</tr>
</tbody>
</table>
left. These levels were also higher in the spermatc veins of both gonads than in the peripheral circulation. No such differences were found in the steroid sulphates except TS which was substantially higher in the right spermatc vein than in peripheral and left spermatc venous blood. Both unconjugated steroids and steroid sulphates were also found in much higher concentrations in the right than in the left gonad. The ratios spermatc to peripheral venous blood varied from 2.6 (DHA in the left) to 118 (T in the right). The levels of all steroids studied were substantially higher in the tissue of both gonads than in the blood from corresponding spermatc veins, except those of A-4 and T in the left gonad, which were similar (A-4) or even lower (T).

The in vitro steroid metabolic pattern observed in the incubated testicular tissue is presented in Table 3. The conversion of [3H]progesterone was expressed as the ratio between its two main metabolites in this in vitro system, i.e. [3H]20α-hydroxy-4-pregn-3-one (20α-DHP) and [3H]17OHP (Berg 1976; Fredricsson & Carlström 1979). The 20α-DHP/17OHP ratio obtained from the right gonad was well within the expected limits for adult men with normal testicular function (Fredricsson & Carlström 1979). The corresponding ratio for the left gonad was extremely high, indicating an immature type of steroid metabolism within this gland. Steroid sulphatase activity, i.e. hydrolysis of oestrone sulphate and DHAS did not differ significantly between the right and the left gonad. However, less oestradiol-17β in relation to oestrone was formed from oestrone sulphate in the left gonad.

Discussion

The syndrome of androgen insensitivity in man is characterized by female external phenotype, scanty or absent sex hair and absence of internal genital ducts in spite of male karyotype and male gonads. The present patient fulfils these criteria. Lack of androgen receptors is accepted to be the cause of the abnormality in rats and mice, as well as in some humans with this syndrome. However, women with

Fig. 4.
Leydig cell adenoma (outlined by arrows) within the left gonad. Stieve's fixative, haematoxylin-eosin stain, × 160.
complete androgen insensitivity have been identified with normal or only qualitatively abnormal androgen receptors (see Griffin & Wilson 1980; Ritzén 1984 for review). These studies are not yet completed in our case.

Much higher levels of LH as compared to FSH have been observed in man (Naftolin et al. 1983) and in the experimental animal (Purvis et al. 1977) characterized by androgen insensitivity. This high LH activity is likely to stimulate gonadotrophin-dependent enzymatic steps involved in testosterone production. A 'mature' type of testicular steroid metabolism in vitro would be expected and has been confirmed in 3 cases of androgen insensitivity (Berg & Kjessler, unpublished data). In the in vitro system here employed a low ratio between 20α-DHP and 17OHP reflects a 'mature' steroidogenesis indicating adequate gonadotrophic stimulation (Berg 1976).

Therefore, there would be no reason to suspect different steroid metabolic patterns to be present in the two gonads of the same individual. Nevertheless, in the present patient they were found to differ considerably with regard to biochemistry as well as to morphology. The 20αDHP/17OHP ratio obtained in tissue from the left gonad indicates an immature type of steroid metabolism and may well fit with previous findings in cases of the incomplete androgen insensitivity syndrome (Bell 1975; Kase & Morris 1965; Morris & Mahesh 1963), while the steroid metabolism observed in the right gonad was more in agreement with that previously found in the complete form of this syndrome.

Oestrone sulphate was less efficiently converted into oestradiol-17β by the left gonad, possibly indicating a less active 17β-hydroxysteroid oxidoreductase activity in this gland. On the other hand we found no major differences in total hydrolysis of oestrone sulphate and DHAS, although it has been suggested that gonadal steroid sulphatase activity may be controlled by gonadotrophins (Domínguez et al. 1974).

The right testis was the main source of gonadal steroids, indicated by steroid levels in spermatic venous blood and in testicular tissue and by the in vitro progesterone conversion patterns. The differences in tissue levels of unconjugated steroids between the two gonads were closely reflected by similar differences in spermatic venous blood. However, this was not the case for the steroid sulphates except for TS. The very high amounts of steroid sulphates in the tissue of the right testis as compared to their levels in spermatic venous blood from the same testis may indicate more steroid sulphate binding proteins in this gonad, as compared to the left. In fact, steroid sulphates are more closely bound to albumin than unconjugated steroids (Crepy & Gueriguinan 1970). Furthermore, binding proteins with medium affinity but high capacity to bind steroid sulphates have been demonstrated in other steroidogenic tissues (Strott & Lyons 1978).

Considering peripheral steroid levels, circulating T, DHT and 17OHP are predominantly of testicular origin in healthy males. On the other hand, P5, DHA and A-4 and also indirectly E3 are mainly of adrenal origin. This also holds true for the corresponding steroid sulphoconjugates (Vermeulen &

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<table>
<thead>
<tr>
<th>Substrate</th>
<th>Right gonad</th>
<th>Left gonad</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20α-DHP/17OHP</td>
<td>0.070</td>
<td>1.772</td>
<td>0.040—0.250</td>
</tr>
<tr>
<td>Substrate [3H]DHAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hydrolysis, fmol x min⁻¹ x mg protein⁻¹</td>
<td>590</td>
<td>1200</td>
<td>—</td>
</tr>
<tr>
<td>Substrate [3H]oestrone sulphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hydrolysis, fmol x min⁻¹ x mg protein⁻¹</td>
<td>1136</td>
<td>1219</td>
<td>—</td>
</tr>
<tr>
<td>Formation of [3H]oestrone, fmol x min⁻¹ x mg protein⁻¹</td>
<td>909</td>
<td>1166</td>
<td>—</td>
</tr>
<tr>
<td>Formation of [3H]oestradiol-17β, fmol x min⁻¹ x mg protein⁻¹</td>
<td>227</td>
<td>53</td>
<td>—</td>
</tr>
<tr>
<td>Oestradiol/oestrone ratio</td>
<td>0.250</td>
<td>0.046</td>
<td>—</td>
</tr>
</tbody>
</table>
Verdonck 1976; de la Torre et al. 1982). The peripheral steroid levels observed in our patient before and after gonadectomy clearly indicate that this was the case here too. Unconjugated DHA only decreased 4 days after surgery but this may be explained by surgical stress effects (Adami et al. 1982).

It is well known that the mean SHBG levels are higher in women than in men. In both sexes SHBG levels decrease considerably during puberty, probably due to increasing androgen levels (Odlind et al. 1982). The high SHBG level found in the present case together with a normal male testosterone level may indicate a lack of androgen responsiveness also in those mechanisms which regulate the synthesis of 'steroid sensitive' liver proteins such as SHBG.

Morphologically the two gonads differed particularly with respect to the amount of connective tissue present. Therefore, proportions between venous and tissue concentrations should be emphasized more than absolute levels. Seminiferous tubules were similarly developed in both. Gonads of patients with androgen insensitivity carry the risk of malignancy, seminomas being most common. However, in this case a number of tubules clearly showed the pattern of carcinoma in situ. Changes of this kind have been reported in 3 out of 8 girls with incomplete androgen insensitivity but none out of 4 girls with the complete form (Müller & Skakkebæk 1984).

To summarize, the features of the right gonad are as would be expected in the complete form of androgen insensitivity, whereas the left gonad presents a type of steroidogenesis found in men with inadequate gonadotrophic stimulation but it also contained much more of DHA and A-4 in relation to T as compared with the other gonad. These findings resemble the situation in the incomplete forms of androgen insensitivity as revealed by other in vitro metabolic studies (Bell 1975). The situation could be explained if the left gonad is deficient with respect to gonadotrophin receptors, or if the adenomatous growth of the right gonad has acquired some degree of steroidogenic autonomy.

The situation could also be explained if the patients has a mosaicism (XY/XXY, the X being the Tfm-carrying X-chromosome). This hypothesis gets some support from varying androgen sensitivity of pubic hair (normal female) and external genitalia (absence of masculinization). However, there are presently no methods available to prove such an hypothesis.

References


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