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Both Reductive Forms of 17β-Hydroxysteroid Dehydrogenase (Types 1 and 3) Are Expressed during Development in the Mouse Testis

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Androstenedione is reduced to form testosterone by 17-β-hydroxysteriod dehydrogenase (17βHSD) and two different reductive isoforms of the enzyme have been identified (types 1 and 3). In this study, levels of mRNA encoding both reductive isoforms have been measured during fetal and post-natal development in the mouse. In fetal and neonatal testes mRNA encoding both type 1 and type 3 isoforms was present at relatively high levels reaching a peak at postnatal day 5. Thereafter, mRNA levels of both 17βHSD isoforms fell to low levels until day 30 when there was a marked increase in the levels of the type 3 isoform. The presence of the type 1 17βHSD enzyme in fetal testes may explain the virilization of the mesonephric (Wolffian) duct which occurs in pseudohermaphrodite individuals lacking the type 3 isoform.

The final step in testosterone biosynthesis is reduction of androstenedione to testosterone by the enzyme 17β-hydroxysteroid dehydrogenase (17βHSD). Five different isoforms of 17βHSD have now been identified in the human, two of which (types 1 and 3) catalyse the reduction reaction while the other three (types 2, 4 and 5) preferentially catalyse the reverse oxidation reaction (1–5). The type 3 isoform was originally isolated from adult human testes and appears to be specific to this tissue (3). Loss of type 3 17βHSD activity in the human testis leads to male pseudohermaphrodism showing that this enzyme is crucial for testicular testosterone synthesis (3). In individuals lacking type 3 17βHSD activity the external phenotype is female although, interestingly, structures derived from the mesonephric (Wolffian) duct are formed normally (3,6,7). This suggests either that androstenedione can stabilise the mesonephric duct or that there is an alternative route to testosterone, perhaps through type 1 activity either in the testis or in the peripheral tissues. In this study we have examined expression of 17βHSD types 1 and 3 during development in the mouse testis and show that type 1 mRNA is present at high levels in fetal and neonatal testes.

MATERIAL AND METHODS

Animals. Normal mice were used in these studies as described previously (8). To time fetal development males were caged with females overnight and the morning was designated as fetal day 0.5 of pregnancy. For studies on post-natal animals the day of birth was designated as day 1. Testes were collected on fetal days 15.5 and 17 and postnatal days 1, 5, 10, 15, 20, 25, 30 and 90 and were frozen and stored in liquid nitrogen until used for RNA preparation.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from testes of individual animals using RNAzol (Biogenesis Ltd, U.K.). RNA was reverse transcribed using random hexamers and M-MLV reverse transcriptase (SuperScript, Life Technologies, U.K.) as previously described (9). The PCRs were carried out in Tris-HCl buffer (75 mM, pH 9.0 at 25°C) containing $(NH_4)_2SO_4$ (20 mM), Tween (0.01%), $MgCl_2$ (2mM), dNTPs (0.2 mM each), *Taq* polymerase (2 U/100 μl), primers (200 nM each) and template (0.1 to 2 μl) in a total incubation volume of 30 μl.

Primers for PCR. Initial primers for PCR amplification of mouse 17βHSD isoforms were designed based on published sequences of human and rat 17βHSD isoforms (1,3,10). Products of these amplifications were sequenced as described previously (11) and shown, by homology, to be derived from mouse 17βHSD types 1 and 3. From these sequences new primers were designed to ensure homology with mouse cDNA and these primers were used for subsequent studies of 17βHSD expression in the mouse testis. Primer sequences:

Type 1 5′-ACT GTG CCA GCA AGT TTG CG-3′ (bases 476–495)
5′-AAG CGG TTC GTG GAG AAG TAG-3′ (bases 788–768)
Type 3 5′-ATT TTA CCA GAG AAG ACA TCT-3′ (bases 365–385)
5′-GGG GTC AGC ACC TGA ATA ATG-3′ (bases 731–711)

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Bases are numbered to correspond to published human sequences (1,3) and products span at least one intron/exon boundary (3,12).

Semi-quantitative RT-PCR. For semi-quantitation of PCR products, identical PCRs were carried out for different cycle numbers over the exponential range of cycles previously determined for each template (13). Each PCR tube contained [^{32}P]dATP (1.5μCi, ICN Biomedicals Ltd, U.K.) and PCR products were separated using agarose gels and counted (11). With each sample β-actin cDNA was co-amplified as an internal control and all values for 17βHSD isoform cDNA are expressed relative to β-actin. Primers for β-actin cDNA were as previously described (14). Controls for each PCR included reverse transcription template with no RNA or with no reverse transcriptase.

RESULTS

Amplification of mouse testis cDNA with primers listed above resulted in single products (Fig. 1) which, when sequenced, were confirmed as being derived from type 1 and type 3 isoforms of 17βHSD. At all ages from fetal day 15.5 both 17βHSD types 1 and 3 were detectable in the mouse testis (Fig. 2) although there appeared to be age-dependent variation in the levels of expression. To measure levels of 17βHSD isoform at each age a semi-quantitative RT-PCR method was used as described above. Quantitative determination using PCR depends upon ensuring that measurements are made during the exponential phase of the amplification. Results in Fig. 3 show data from one experiment in which 17βHSD types 1 and 3 were measured relative to β-actin in a day 5 testis. Accumulated results from experiments to measure 17βHSD isoforms during development are shown in Figure 4. During fetal and early neonatal life both type 1 and type 3 isoforms are expressed at about equal levels reaching a peak at postnatal day 5. Thereafter, there is a decline in both isoforms to barely detectable levels at day 25 followed by a marked increase in the type 3 isoform between days 25 and 30. The increase in type 3 isoform continues into adulthood while the type 1 isoforms remains at low prepubertal levels.

DISCUSSION

Results described here show that levels of mRNA encoding both reductive forms of 17βHSD are high during fetal and neonatal development but that there is a marked shift to the type 3 isoform at puberty. The type 3 isoform is known to have a high affinity for androstenedione and reduced affinity for estrone (3). The type 1 enzyme, in contrast, has a high affinity for estrone but activity is reduced to 20% with androstenedione as substrate (15). The presence of high levels of 17βHSD type 1 enzyme in fetal and neonatal testis may be related, therefore, to estrogen production during this time. Several studies have reported that fetal and neonatal rat testes contain aromatase activity (16,17) and it has been shown recently that cytochrome P450 aromatase mRNA is present in fetal mouse testes at levels apparently higher than in fetal ovaries (18). The possible function of testicular estrogen during this period is unknown although estrogen receptors are present in fetal testes and associated ducts (19).

The role of the type 3 enzyme during testis development is clear. Testosterone is required during

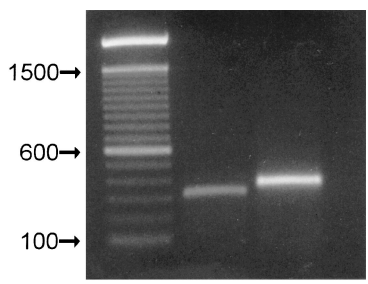

FIG. 1. Amplification of 17βHSD types 1 and 3 by PCR. RNA from testes of a day 30 mouse was reverse-transcribed and amplified using primers described in the text. Lane 1 contains a 100bp-ladder.

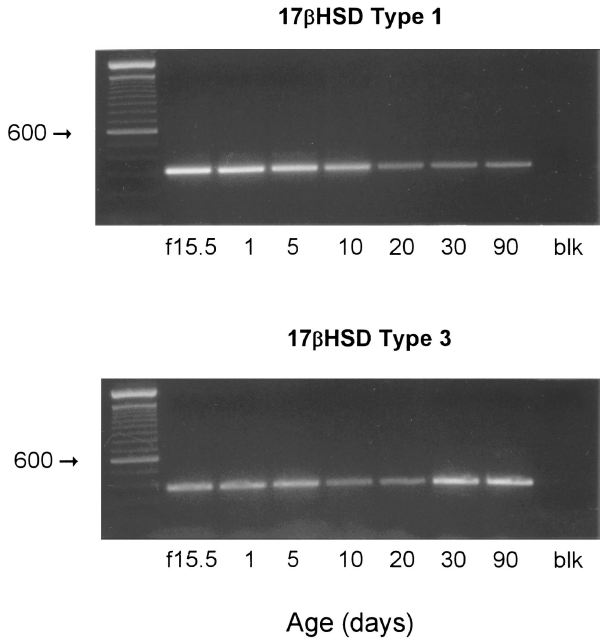

FIG. 2. Amplification of 17βHSD types 1 and 3 by RT-PCR using RNA from testes of mice of different ages. Position of the 600bp marker is shown. f = fetal.

male fetal development to induce formation of mesonephric duct derivatives and to act as a precursor of dihydrotestosterone which masculinizes the external genitalia (20). In individuals lacking type 3 17βHSD activity the external genitalia fail to masculinize but mesonephric duct derivatives form as normal (3,6,7). Data shown here offer a possible explanation of this apparent contradiction. Expression of type 1 17βHSD in the fetal testis would allow conversion of andro-

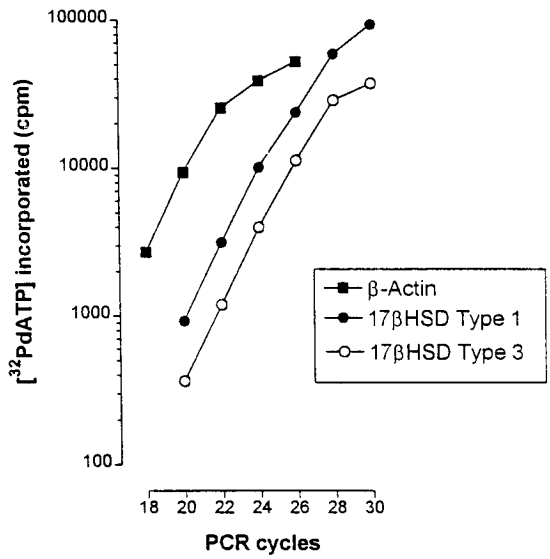

FIG. 3. Semi-quantitative measurement of 17βHSD types 1 and 3 relative to β-actin using RT-PCR. RNA was extracted from testes of a day 5 mouse and cDNA amplified as described in the text. Incorporation of [^{32}P]dATP is shown over different numbers of PCR cycles.

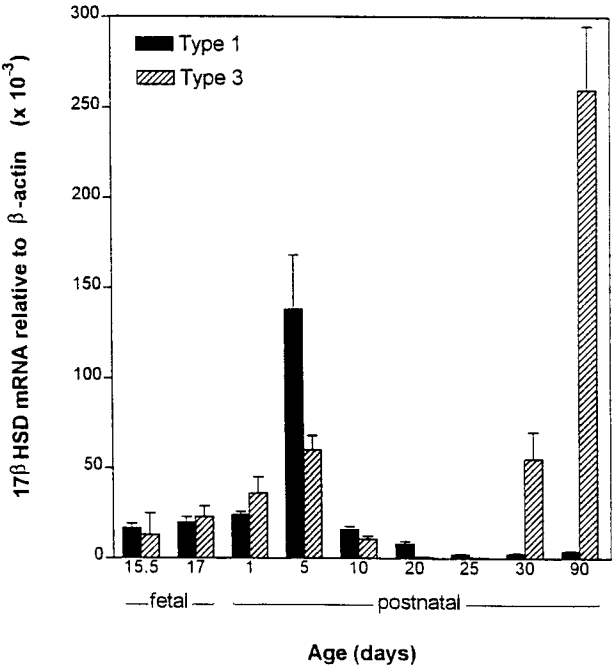

FIG. 4. Accumulated results showing 17βHSD types 1 and 3 measured in individual animals from fetal day 15.5 to postnatal day 90. Each group represents the mean ± sem of 3 or 4 animals.

stenedione to testosterone but the levels of testosterone produced would be low because of the low affinity of the type 1 enzyme for C_{19} steroids. This may be enough to virilize the mesonephric duct but not enough for significant conversion to dihydrotestosterone by the external genitalia. Evidence from humans with partial androgen insensitivity or rats treated *in uteri* with anti-androgen suggest that development of the male external genitalia is more sensitive to changes in normal androgen action than the mesonephric ducts (21,22). Conversion of androstenedione to testosterone by testicular 17βHSD type 1 will be significantly greater than peripheral conversion by the type 1 enzyme because local concentrations of substrate will be markedly higher.

Unlike other testicular enzymes involved in androgen biosynthesis 17βHSD activity in the adult animal is not confined solely to the Leydig cells (23). It is uncertain, therefore, in which cells type 1 and type 3 17βHSD mRNA is expressed during fetal and early neonatal development. The pattern of expression would fit most closely with development of the fetal Leydig cells which begin to be superseded by the adult type around 10 days in the mouse (24). If fetal Leydig cells express both type 1 and type 3 isoforms of 17βHSD whereas adult Leydig cells express only the type 3 isoform this would explain the pattern of expression in the testis. Changes in postnatal expression of type 3 17βHSD mirror changes in testicular 17βHSD enzyme activity reported previously with a marked change in both enzyme activity and mRNA levels between 25 and 30 days (25). The period between 25 and 30 days is known to be crucial for normal adult Leydig cell functional development (25,26).

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