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Differentiation-Dependent Expression of 17β-Hydroxysteroid Dehydrogenase, Type 10, in the Rodent Testis: Effect of Aging in Leydig Cells

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Expression of the new 17β-hydroxysteroid dehydrogenase (HSD), type 10 (17β-HSD-10), formerly known as endoplasmic reticulum-associated amyloid-binding protein, has been investigated in the testes of various mammals under normal and perturbed conditions. Results show that 17β-HSD-10 is a major product of both fetal and adult-type Leydig cells. In the former, protein persists until late in postnatal development; and in the short-day hamster model, it does not disappear when Leydig cells involute. During puberty in the rat, immunohistochemical staining for 17β-HSD-10 in adult-type Leydig cells first becomes evident on d 20, increasing to maximal staining intensity by d 35. In the rat, but not in the mouse or any other species examined, there is also staining in late spermatids. Examination of testes from rats subjected to perinatal treatment with either a GnRH antagonist or low and high doses of diethylstilbestrol revealed that expression of 17β-HSD-10 follows closely Leydig cell differentiation status, correlating with 3β-HSD expression in a previous study. In aging (23 months) rat testes, Leydig cell (but not germ cell) immunostaining for 17β-HSD-10 is markedly reduced. 17β-HSD-10 seems to preferentially convert 3α-androstanediol into dihydrotestosterone, and estradiol to estrone. Thus, perinatal expression of this enzyme in fetal Leydig cells may contribute to protecting these cells from estrogens and encourage androgen formation. (*Endocrinology* 144: 3130–3137, 2003)

CIRCULATING AND LOCAL steroid concentrations are determined by a balance between synthetic and catabolic processes. Whereas, in the context of androgen production in the male, there has been considerable research on the generation of steroids by the testicular Leydig cells, and their subsequent conversion by 5α-reductase, in target tissues, to the more potent dihydrotestosterone (DHT), comparatively little is known about their catabolism to less active metabolites. Within the testis, the local action of catabolic enzymes like the 17β-hydroxysteroid dehydrogenases (17β-HSDs), type2 and type 4, may be important to protect testicular cells from excessive androgen levels (1, 2). Together with steroid-binding globulins, these enzymes supposedly regulate the amounts of androgens available to activate intratesticular androgen receptors.

Recently, a new member of the short-chain alcohol dehydrogenase family was described, originally in the context of Alzheimer disease, as a brain factor (endoplasmic reticulum-associated amyloid-binding protein, ERAB) linked to neurotoxicity (3). Further biochemical characterization of the recombinant enzyme showed it to be a novel member of the large family of 17β-HSDs now referred to as 17β-HSD, type 10 (17β-HSD-10) (4–7). Interestingly, although estradiol and other 17β-hydroxysteroids can act as substrates with 17β-HSD-10, removing estradiol approximately as effectively as type 4 17β-HSD (7), by far the highest affinity is seen for 3α-androstanediol (3α-diol), converting this efficiently into DHT (5, 7). Thus, the principal role for such an enzyme, besides removing estradiol, could be to protect DHT from catabolism to less active metabolites. Unlike other 17β-HSD members, 17β-HSD-10 seems to be a mitochondrial protein (7), though encoded by a nuclear gene (human, chromosome Xp11.21; mouse, chromosome 9) and thus, when expressed in steroidogenic cells, is likely to influence steroidogenesis at source. The gene for 17β-HSD-10 encodes a 27-kDa protein, which *in vivo* forms 108-kDa homotetramers (7). It seems to be a multifunctional enzyme and, like other members of the short-chain hydroxyacyl-coenzyme A (CoA) dehydrogenase family, also exhibits fatty acid β-oxidation properties (4, 7), which may be physiologically relevant, given its presumed mitochondrial location.

From an earlier differential cloning project, we were able to show that gene transcripts for 17β-HSD-10 (previously referred to as ERAB) were highly expressed in the mouse testis (8) and seemed to be preferentially up-regulated in the Leydig cells of the azoospermic w/w^v mouse. The localization in Leydig cells was indirect, being deduced from an enrichment of the transcript hybridization by Northern blotting in primary Leydig cells, compared with intact testis. In the present study, we have made use of a newly available antibody, raised against 17β-HSD-10, to make a detailed study of the expression of this enzyme in the testes of different mammals, at different times of development and under different perturbation regimes, including also the effect of ag-

Abbreviations: CoA, Coenzyme A; DES, diethylstilbestrol; DHT, dihydrotestosterone; 3α-diol, 3α-androstanediol; ERAB, endoplasmic reticulum-associated amyloid-binding protein; hCG, human chorionic gonadotropin; HSD, hydroxysteroid dehydrogenase; *hpg*, hypogonadal; 17β-HSD-10, 17β-HSD (type 10); RLF, relaxin-like factor.

ing, with a view to reaching an understanding of its possible role in male reproductive function.

Materials and Methods

Animals and tissues

Adult testis tissues were obtained from wild-type NMRI (Charles River Laboratories, Inc., Sulzfeld, Germany), hypogonadal (*hpg*), and w/w^v mice (The Jackson Laboratory, Bar Harbor, ME); from Djungarian hamsters that, following normal daylight cycles, had been subjected to either short (8-h light) or long (16-h light) daylight regimes (courtesy of Dr. Alexander Lerchl, Münster, Germany); from adult marmoset monkeys (courtesy of Dr. Stefan Schlatt, Münster, Germany); and from human specimens obtained at orchidectomy for prostate carcinoma, as previously described (9). Authorization to use human tissue samples had been obtained from the local ethical committee, and the requirements of the Helsinki convention were observed in all cases. All tissues were either frozen immediately in liquid nitrogen and stored at −80 C until used for extraction of RNA (see below), or were rapidly immersion-fixed in Bouin's solution, following standard procedures (10). Untreated rat tissues were obtained from Wistar rats (Charles River Laboratories, Inc.) at indicated times after birth. Animals were killed by cervical dislocation, immediately after CO_2 anesthesia, and tissues were treated as above. For comparing young adult (3 months) and old (23 months) tissues, Wistar rats were used and, where indicated, the fresh testicular tissue was carefully dissected into tunica albuginea, seminiferous tubules (free of interstitial cells), and testicular blood vessels. Rat Leydig cells were prepared by enzymatic digestion as previously described (11) and used immediately either for protein extraction, for Western blotting, or for RNA extraction. In addition to these untreated rat tissues, testicular tissue sections were also used from Wistar rats subjected to perinatal treatments with either a long-acting GnRH antagonist (Antarelix; Europeptides, Argenteuil, France; sc injection of 10 mg/kg in 20 μl 5% mannitol on d 2 and 5) or with differing doses (0.1 or 10.0 μg in 20 μl corn oil at 2-d intervals, delivered sc from postnatal d 2–12 inclusive) of the estrogen diethylstilbestrol (DES; Sigma, Poole, UK), as previously described (12, 13). Mouse Leydig cells were prepared, from 3-month-old NMRI mice, by mechanical dispersion only, followed by Percoll gradient centrifugation (14). Immunocytochemistry of the fresh and cultured mouse Leydig cells for the Leydig cell-specific marker relaxin-like factor [RLF, or Insl3; (10)] indicated a purity of more than 95% (not shown). For Western analysis (see below), the primary mouse Leydig cells were cultured for 3 h with either vehicle, 1 mM 8Br-cAMP, or 5 ng/ml human chorionic gonadotropin (hCG) (both from Roche Molecular Biochemicals, Mannheim, Germany) in DMEM (Sigma, without sodium bicarbonate but with L-glutamine and 15 mM HEPES) containing 14.3 mM sodium bicarbonate, 0.05% (wt/vol) BSA (fraction V; Merck KGaA, Darmstadt, Germany), and 10 ml/liter penicillin-streptomycin solution (Sigma) (10,000 U/ml penicillin; 10 mg/ml streptomycin). All animal experimentation was carried out with appropriate authorization from the local ethical committees.

RNA extraction and analysis

Rat tissues and cells were extracted by a one-step procedure, using a phenol-free, filter-based RNA isolation system (RNAqueous) according to the instructions of the manufacturer (AMS Biotechnology, Wiesbaden, Germany). RNA electrophoresis was carried out using the standard formaldehyde/4-morpholinepropanesulfonic acid procedure (15), and RNA was transferred by 4 h capillary transfer to nylon membranes (Nytran; Schleicher & Schuell, Inc., Dassel, Germany). These were hybridized, again using standard procedures (15), to a radioactively labeled full-length cDNA insert of the mouse 17β-HSD-10 (ERAB) gene, exactly as previously described (8). Even loading of RNA samples was checked by assessment of the ethidium bromide staining of the 28S ribosomal RNA band. Additional Northern blots were performed comparing whole testis, seminiferous tubules, and percoll-purified Leydig cells from young and old rats, loading 12 μg total RNA from each of three independent samples in each category, together on single gels. Each Leydig cell sample comprised a pool from four different animals. For quantification of the autoradiographs, these were densitometrically scanned and subjected to analysis using ImageQuant 5.0 software (Amersham International Biosciences, Freiburg, Germany), normalizing using the intensity of the ethidium bromide-stained 28S ribosomal RNA band. Results were expressed as arbitrary units, setting the mean of the values for cells and tissues from young rats at 100. Data are expressed as means ± SDs, and statistical significance is estimated by Student's *t* test.

Protein extraction and immunological analysis

For Western blotting, Leydig cells were washed once with cold PBS and homogenized in cell lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_2VO_4, 1 μg/ml Leupeptin, and 1 mM PMSF (Cell Signaling Technology, Beverly, MA). Ten-percent tissue homogenates were also prepared in the same cell lysis buffer. Protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Inc., München, Germany). Equal amounts (40 μg) of the resulting protein extracts were subjected to SDS-PAGE in 10% gels and transferred to positively charged polyvinylidene difluoride membranes (Amersham International Biosciences) for 2 h at 168 V. Membranes were blocked with buffer containing 1× Tris-buffered saline, 0.1% Tween-20, and 5% nonfat dry milk and incubated at room temperature for 1 h. For immunodetection of 17β-HSD-10, membranes were incubated for 24 h at 4 C with rabbit antihuman 17β-HSD-10 affinity-purified antibody (BioTrend Chemicals, Köln, Germany) diluted 1:1000. This antibody had been shown by the manufacturer to specifically recognize a 14-amino-acid peptide sequence (designated ERAB12) from the middle portion of mouse ERAB (17β-HSD-10), a sequence that is 100% identical with the equivalent region of the human sequence. Membranes were then washed, incubated with goat antirabbit horseradish peroxidase-conjugated antibody (1:2000) and antibiotin antibody (1:1000), both obtained from Cell Signaling Technology, for 1 h at room temperature. After washing, the signal was detected using the Lumiglo reagent (Cell Signaling Technology). To check the loading of the samples, membranes were stripped, washed, blocked with blocking buffer (as above), and incubated overnight at 4 C with monoclonal anti-β-actin antibody (Sigma) diluted 1:8000. The membrane was washed, and then incubated at room temperature for 1 h with horseradish peroxidase-linked antimouse IgG (1:5000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and the signal was detected using chemiluminescence reagent (Pierce Chemical Co. Biotechnology, Rockford, IL). As for the Northern blots, Western blots were also repeated for triplicate independent samples of whole testis, seminiferous tubules, and Leydig cells from young and old rats, with the Leydig cells being derived from 4 rats for each sample (40 μg protein was loaded per sample). Specific signals were quantified using ImageQuant software, normalizing using the β-actin chemiluminescent intensity, and expressed as arbitrary units, with the mean of the values for the cells and tissues from young rats set at 100. Data are expressed as means ± SDs, and statistical significance is estimated by Student's *t* test.

Immunohistochemistry of 6- to 8-μm sections of Bouin's-fixed tissues, embedded in paraffin, followed the method described in Balvers *et al.* (10), using a double PAP-ABC (phosphatase antiphosphatase–avidin-biotin-complex) procedure. For most tissues, the primary antibody (see above) was applied at a dilution of 1:100. As negative control, an equivalent amount of control rabbit IgG (Vector Laboratories, Inc., Burlingame, CA) was applied. For the semiquantitative assessment of epitope intensity in the rats subjected to various perinatal treatment regimes, preliminary experiments were carried out to show that, even for the highest local concentration of epitopes, the primary antibody was not saturating, and hence, a gradation of staining intensity could be obtained. All final immunohistochemistry was carried out in parallel under identical conditions. For these sections, 17β-HSD-10 protein expression was estimated by counting all hemalum-stained interstitial cell nuclei (including Leydig cells and their precursor mesenchymal cells, fibroblasts, and macrophages, but excluding endothelial cells) within a defined frame of view (0.4×0.5 mm^2), for three randomly chosen frames per tissue section and animal, for a total of three to four animals per experimental data-point and, of these, counting those with clearly positive immunostaining for 17β-HSD-10 epitopes. Results were expressed as the percentage (mean ± SD) of positively staining interstitial cells. In addition, a subjective estimate of staining intensity of positive cells was made on a scale of: 0, no staining; I, just detectable staining; II, moderate

staining; to III, heavy staining. Where groups of fetal Leydig cells were still readily identifiable in the postnatal rat testis, these were also noted. All sections were blinded before assessment by an independent observer.

Results

Expression of 17β-HSD-10 in Leydig cells

Confirming what had been suggested previously only at the mRNA level (8), the application of a specific antibody raised against 17β-HSD-10 indicates the expression of the protein almost exclusively in the Leydig cells of the adult testes of most mammalian species analyzed (Fig. 1). Comparing different mouse mutants shows that 17β-HSD-10 is expressed in the Leydig cells of the w/w^v azoospermic mouse, just as in wild-type mice, but not in the Leydig cells of the *hpg* azoospermic mouse, which, lacking a functional hypothalamo-pituitary-gonadal axis, are arrested at a prepubertal

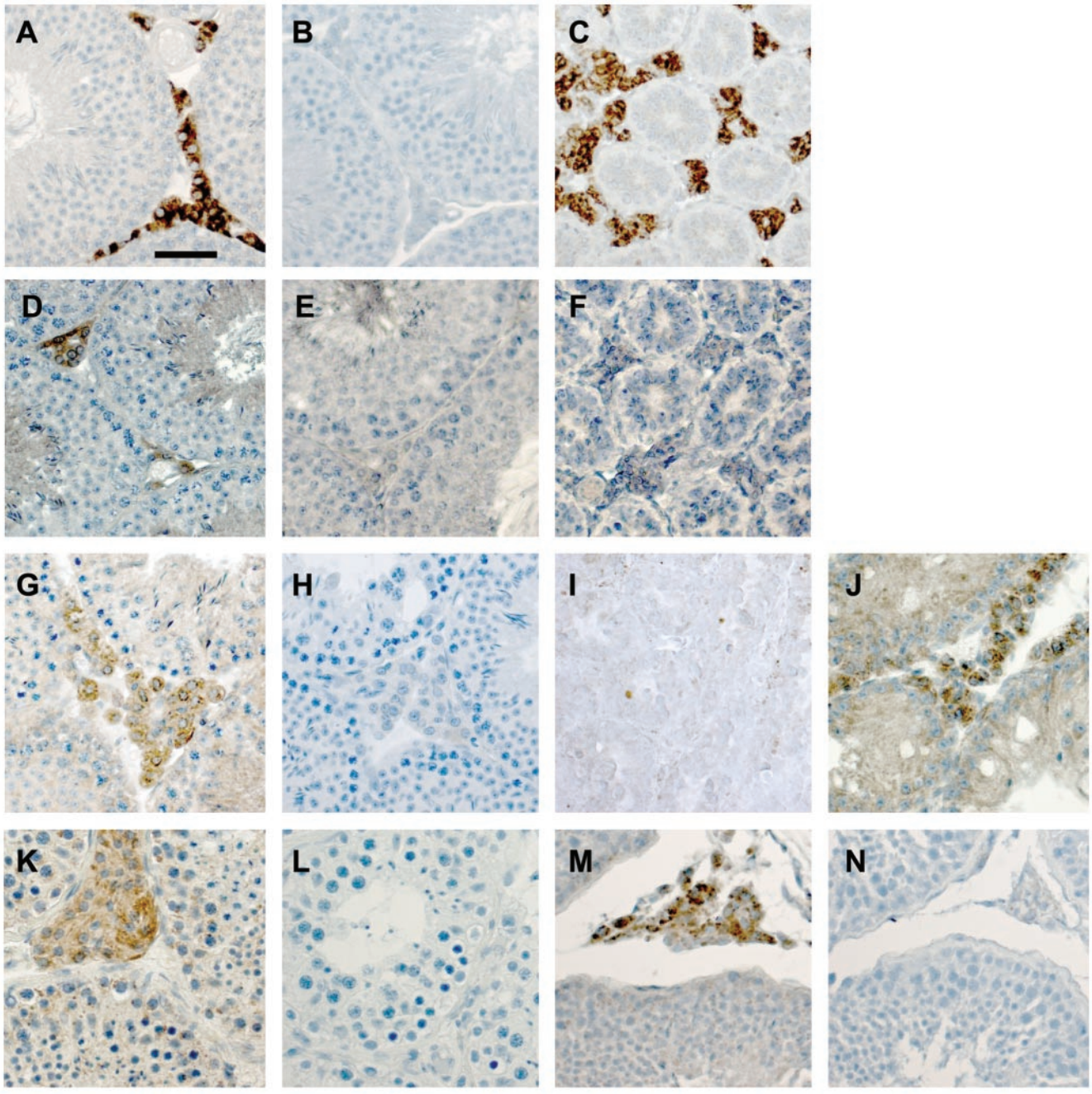

FIG. 1. Immunohistochemistry for 17β-HSD-10 in the testes of various mammals: A, hamster (long day); B, hamster (long day), control; C, hamster (short day); D, hamster (long day), RLF immunostaining; E, hamster (long day), RLF control; F, hamster (short day), RLF immunostaining; G, adult mouse; H, adult mouse, control; I, adult *hpg* mouse; J, adult w/w^v mouse; K, human testis; L, human testis, control; M, adult marmoset testis; N, adult marmoset testis, control. All controls represent parallel sections incubated with equivalent amounts of preimmune IgG (17β-HSD-10) or the preimmune serum from the subsequently immunized animal (RLF/Insl3). All sections are at the same magnification (*scale bar* in A, 50 μm).

stage. The results from the hamster testis (Fig. 1) are instructive; not only are the Leydig cells from the spermatogenically active long-day testis highly stained for 17β-HSD-10, but also those from involuted testes of animals kept in a short daylight regime. This is in contrast to another marker of Leydig cell function, the relaxin-like factor (RLF/Insl3), whose expression reflects the differentiation status of the Leydig cells in this species (Fig. 1).

Developmental expression of 17β-HSD-10 in the rat testis

In a detailed study of 17β-HSD-10 expression in the rat testis from birth to adulthood (Fig. 2), first it was observed that the fetal Leydig cells stain heavily and can thus be easily followed through postnatal development at least up to d 15. Adult interstitial cells only show light staining during puberty (d 20–25), and they first show moderate to heavy staining intensity on about d 30–35, which persists into adulthood (Fig. 2, Table 1). Treatment of rats postnatally with a GnRH antagonist to block the hypothalamo-pituitary-gonadal axis, and hence retard puberty and reduce the numbers of Sertoli cells (12), does not lead to any major change in the proportion of positively staining Leydig cells at d 35 and in adulthood (Table 1), but there was a significant reduction in positively stained cells at earlier stages. This is attributable partly to the block in pubertal development of the adult generation of Leydig cells caused by the GnRH antagonist (*e.g.* d 25; see Ref. 13) but also to a failure to detect fetal Leydig cells on d 18. In these experiments, no attempt was made to assess absolute numbers of Leydig cells, which are a complex function of growth and differentiation of adult-type Leydig cells, and of the other testicular cell components (16), but only the percentage of interstitial cells expressing 17β-HSD-10 epitopes. The results are therefore to be judged only in comparison with the control group.

Treatment of rats perinatally with either low-dose or high-dose estrogen regimens led to opposing effects. The low-dose (0.1 μg) DES treatment led to an apparent advancement of 17β-HSD-10 expression in Leydig cells (Table 1). In contrast, the high-dose (10.0 μg) DES treatment seemed to have minimal effect on the early Leydig cell population but markedly retarded the development of 17β-HSD-10 staining intensity in the adult population of Leydig cells. The latter finding may indicate a preferential effect of high levels of DES on 17β-HSD-10 in adult (rather than fetal) Leydig cells.

Unlike the other mammals that have been assessed, rats uniquely also exhibit marked immunostaining for 17β-HSD-10 within the seminiferous tubules, in the postmeiotic stages of spermiogenesis (Fig. 2). This does not seem to be a staining artifact, because both Northern (Fig. 3) and Western (Fig. 4) analyses confirm the presence of the mRNA and a weak 27-kDa protein, respectively, in rat tubules. Nevertheless, both in the rat and the mouse, within the gonad, the Leydig cells seem to be the major site of the enzyme synthesis, because both mRNA and protein are greatly enriched in primary Leydig cell preparations, compared with whole testis.

The Western analysis thus also confirmed the specificity of the antibody used, which consistently indicated a 27-kDa protein in those tissues (*e.g.* testis and liver) from which expression has previously been reported (7, 8, 17). Western analysis also showed that 17β-HSD-10 does not seem to be acutely up-regulated in mouse Leydig cells by the classic effectors 8Br-cAMP or hCG (Fig. 4).

Effect of aging on 17β-HSD-10 expression in the rat testis

Immunohistochemical assessment of rat testis sections for 17β-HSD-10 expression showed that there is a massive reduction in staining intensity in the Leydig cells of old (23 months), compared with young (60 d), rat testes (Fig. 2). There seems to be no effect of age on the expression of 17β-HSD-10 within the seminiferous tubules. This was further analyzed by Northern and Western analyses. In the rat, no differences were seen in the hybridization intensity of 17β-HSD-10 transcripts between young (3 months) and old (23 months) rats for a variety of tissues (heart, adrenal glands, testes) and testicular components (tunica albuginea, seminiferous tubules; testicular blood vessels) (Fig. 3). However, comparing young and old Leydig cells did indeed show that the former had a significantly greater hybridization intensity ($P < 0.001$; Fig. 5). Western blotting gave similar results (Figs. 4 and 5): whereas triplicate samples of whole testis and seminiferous tubules showed no significant effect of age on the amounts of immunoreactive protein (data not shown), for Leydig cells there was a marked difference ($P < 0.001$) between young and old rats in the staining intensity of the specific 27-kDa band (Fig. 5).

Discussion

The enzyme 17β-HSD-10 is a mitochondrial component that seems to have a preference for 3α-diol as substrate, followed by estradiol and other 17β-hydroxysteroids (4, 5). Here, we show that it has a highly conserved expression in the Leydig cells of all mammals so far tested, confirming at the protein level what was suggested previously at the mRNA level for the mouse (8). These results are additionally supported by a recent study in which 17β-HSD activity, with a preference for 3α-diol as substrate and high expression in fetal Leydig cells, was shown enzyme-histochemically in the Leydig cells of the rat testis (18). The present study shows first that 17β-HSD-10 is an important product of the fetal population of Leydig cells, continuing to be expressed at a time-point postnatally when functionally the fetal Leydig cells have begun to involute, no longer producing large amounts of steroids (19, 20). Indeed, it would seem that 17β-HSD-10 could be a useful marker for tracing the fate of this population of Leydig cells through postnatal development, with the evidence here supporting the notion that these cells do not all enter apoptosis and degenerate (21) but can persist in later puberty as discrete clumps (22, 23). This apparent persistence of 17β-HSD-10 expression in involuting Leydig cells is also observed in the adult hamster testis (Fig. 2), after a shift in light regime to short days. In the short-day hamster testis, the Leydig cells otherwise dedifferentiate, losing their steroidogenic capacity (24), and even their ability to express the constitutive marker, RLF/Insl3 (Fig. 1).

In the adult population of Leydig cells, 17β-HSD-10 begins to be expressed at about d 20–25 in the postnatal rat, increasing in immunostaining intensity up to adulthood. Post-

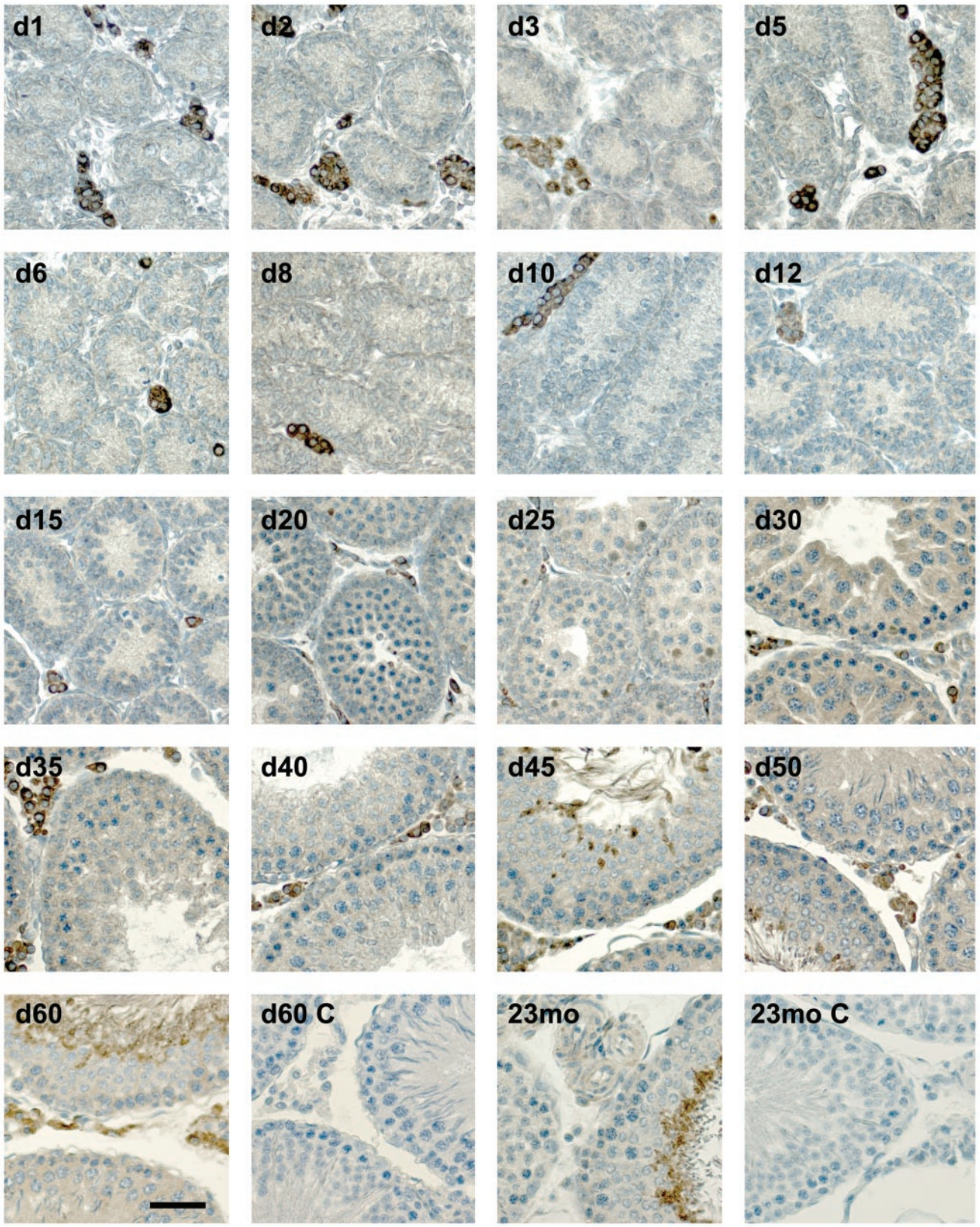

FIG. 2. Immunohistochemistry for 17β-HSD-10 in the postnatal rat testis from birth to adulthood (d 1–60) and in old age (23 months). Note the appearance of germ-cell staining from d 45 onwards. All positively stained cells from d 1–15 are presumed to be fetal Leydig cells. C, Negative control of parallel sections incubated with an equivalent amount of preimmune IgG instead of the primary antibody. All sections are at the same magnification (*scale bar* in *bottom left*, 50 μm).

TABLE 1. Percentages of interstitial cells (haemalum-stained nuclei) immunopositive for 17β-HSD-10 epitopes

Stage	Control	GnRH antagonist	DES 0.1 μg	DES 10.0 μg
d 3/4	1.98 ± 2.81 (0/F)	n.d.	n.d.	3.57 ± 1.35 (0/F)
d 10	2.01 ± 1.87 (0/F)	n.d.	n.d.	0.16 ± 0.23 (0/F)[a]
d 18	3.35 ± 2.29 (I/F)	0.79 ± 1.02 (I)[a]	2.71 ± 1.95 (I)	0.78 ± 1.18 (I/F)[a]
d 25	5.84 ± 7.32 (I)	0.03 ± 0.09 (I)[a]	18.29 ± 8.83 (I/II)[a]	2.15 ± 3.46 (I/F)
d 35	68.88 ± 29.98 (II)	78.30 ± 15.98 (II)	73.01 ± 14.60 (III)	0.34 ± 0.29 (I/II)[a]
d 90	95.54 ± 2.26 (III)	93.99 ± 2.24 (III)	91.54 ± 6.66 (III)	57.23 ± 39.06 (II)[a]

Data are means ± SDs (n = 9–12; 3 random fields from 3–4 animals). n.d., Not determined.

[a] Significant differences from the control group ($P < 0.05$; Student's t). *In parentheses* is indicated the average intensity of staining, where positive, in most fields of view, of interstitial cells that were not identifiable as fetal Leydig cells: 0, no staining; I, staining just detectable; II, moderate staining; III, heavy staining; F, the presence amongst the immunopositive cells of clearly identifiable groups of fetal Leydig cells. With minor exceptions, the relative changes in the proportion of interstitial cells immunopositive for 17β-HSD-10 shows a strong parallel to the numbers of 3β-HSD immunopositive cells reported earlier in the same animals (13).

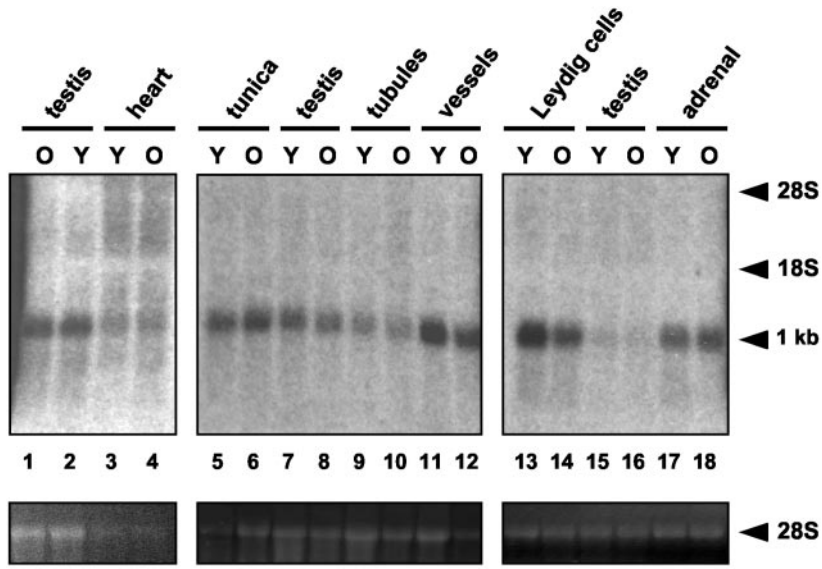

FIG. 3. Northern hybridization of total RNA from young (Y) and old (O) rat cells and tissues, as indicated. The *right-hand panel* was exposed to autoradiography film for a shorter time than in the other panels. Ethidium bromide staining of the 28S ribosomal RNA was used as control for equivalent RNA loading for young and old tissues. Testis samples from young and old animals are from different individuals. Leydig cell, seminiferous tubule, tunica, and blood vessel samples were pooled from four animals in each category. RNA loading: lanes 1 and 2, 15 μg; lanes 3 and 4, 6 μg; lanes 5–18, 10 μg. Note that only Leydig cells indicate an age-dependent difference in signal intensity.

natal treatment with a GnRH antagonist causes a delay in Leydig cell growth and differentiation, reflected by reduced testosterone production (13), but Leydig cell numbers and differentiation catch up by adulthood (13). The appearance of 17β-HSD-10 immunostaining reflects this delayed onset of cell differentiation, though catching up by d 35. Treatment of rats postnatally with low or high doses of estrogens is known to perturb Leydig cell growth and differentiation in a dose-dependent manner (12, 13, 25). As shown here, at the higher dose of DES, the expression of 17β-HSD-10 accurately reflects the DES-dependent retardation of adult Leydig cell numbers and function (13). Interestingly, at the lower dose of DES, whereas there is no apparent influence of the estrogen on the final intensity of 17β-HSD-10 expression on d 35 and 90, there was a significant induction of 17β-HSD-10 expression on d 18. This correlates with an observed increase in circulating testosterone at this time in these animals (13), and perhaps indicates some advance in the functional differentiation of the adult Leydig cell population. In primary cell culture, 17β-HSD-10 expression does not seem to be affected by acute (3-h) stimulation with the effectors 8Br-cAMP or hCG, thus excluding a rapid regulation like that for steroidogenic acute regulatory gene transcripts. Though longer incubation times were not tested, these results suggest that the 17β-HSD-10 gene is probably regulated in a differentiation-dependent manner, rather than acutely by hormonal influences.

The rat seems to be atypical, in that 17β-HSD-10 immunoreactivity is also observed in postmeiotic germ cells within the seminiferous tubules. Because this is seen neither in the mouse, hamster, nor any other mammal studied, this result should be regarded with caution. This is presumably an evolutionarily recent event and may be without physiological significance. Such an observation would reinforce the views of those proponents of the notion that much postmeiotic gene expression is not of functional significance but rather reflects promiscuous transcription of the unwinding haploid genome (26). Alternatively, it may be significant that, according to the Western blot analysis, the absolute concentrations of 17β-HSD-10 in the whole rat testis are markedly lower than those in the mouse or in the human gonad, and that possibly the *de novo* expression in the seminiferous tubules is an attempt to redress this deficit. Given that aromatase can also be detected in postmeiotic germ cells of both the rat and the human (27, 28), 17β-HSD-10 is possibly involved in regulating estradiol in this testicular compartment (see below). 17β-HSD-10 is also expressed in other organs of the body, including the liver, brain, adrenal gland, skeletal muscle, lung, and heart (7, 8, 17); and hence, it is not unexpected to detect expression also in blood vessels and in the

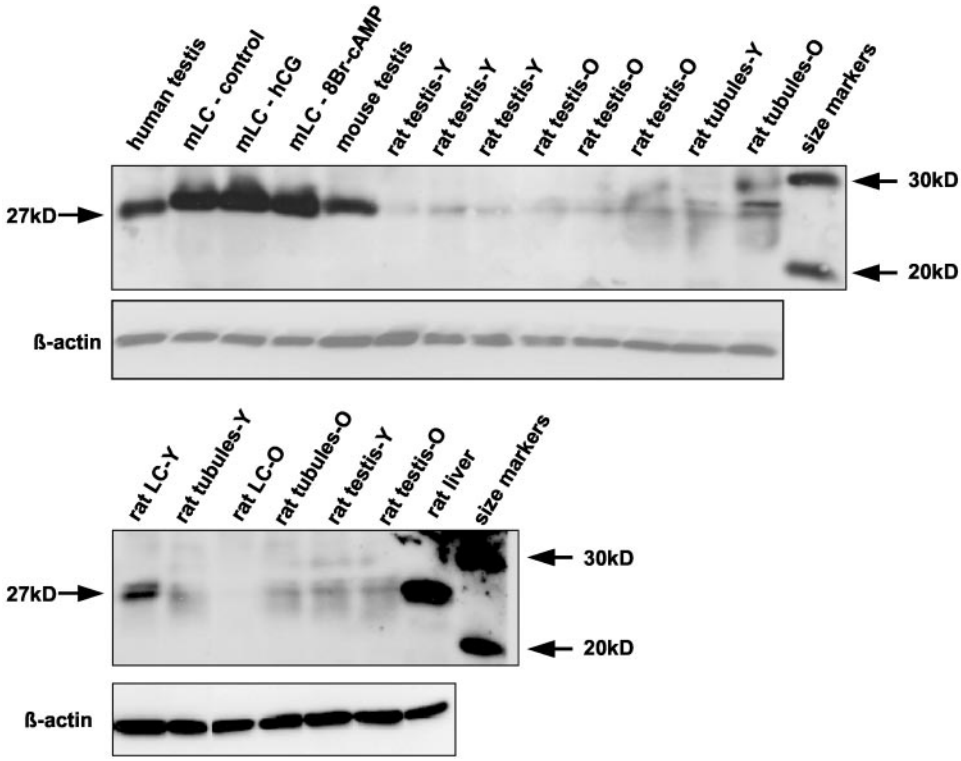

FIG. 4. Western blot analysis of 17β-HSD-10 in various testicular cells and tissues, as indicated [Y, young (90 d); O, old (23 months)]; and in a positive control tissue, rat liver. Forty micrograms of protein was loaded in all lanes. β-actin immunostaining of the same stripped blots was used as loading control. Note that there is no significant difference between signal strengths of whole testis, and seminiferous tubules between young and old rats, yet a marked difference between Leydig cells (LC) from young and old rats. Rat LC and seminiferous tubule samples were pooled from four animals in each category. Also, there seem to be two immunostained bands in samples from rat seminiferous tubules, compared with other cells and tissues. Acute treatment of cultured primary mouse LC (mLC) with hCG or 8Br-cAMP showed no influence on staining intensity.

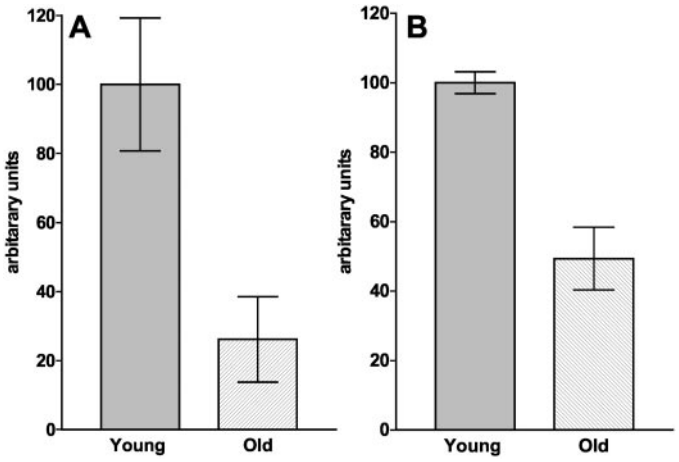

FIG. 5. Densitometric evaluation of 17β-HSD-10 protein by Western blotting (A) and mRNA by Northern blotting (B) for percoll-purified Leydig cell preparations from young and old rats, as indicated (means ± SDs; n = 3).

tunica albuginea of the rat testis. However, based on the immunohistochemical results, the contribution from these somatic components to enzyme activity in the testis is likely to be relatively small, compared with that of the Leydig cells.

In the male, aging is associated with a decline in circulating androgen levels, attributable to a decrease in the steroidogenic capacity of the testis; in particular, of the Leydig cells (29). Most studies on the aging male have focused on steroid biosynthesis. Here, we have been able to assess the influence of age on a quantitatively important steroid metabolizing enzyme and have shown, for the rat, that there is a marked decrease in transcript level, but most especially in protein level, assessed both by Western blot and by immunohistochemistry, of 17β-HSD-10. This effect is very specific to Leydig cells, because it was observed in no other testicular component or for any other organ examined.

17β-HSD-10 is probably a multifunctional enzyme, with both 17β-HSD and acyl-CoA dehydrogenase properties (6, 30). It is located in the mitochondria, also of Leydig cells (not shown). Given this location and possible functions, then, in Leydig cells, it would seem to be an important synthetic enzyme encouraging the formation of DHT from its precursor and degradation metabolite 3α-diol. It could also, though to a lesser extent, convert androsterone to androstanedione (5). Theoretically, these pathways would all act to increase the androgenic potential of the Leydig cells; and conversely, its reduction in old age would exacerbate any age-associated hypoandrogenemia. 17β-HSD-10 thus seems to have a role similar to that of the type 2 retinol dehydrogenase, which is also present in Leydig cells (31). 17β-HSD-10 is also able preferentially to convert estradiol to its inactive metabolite estrone (6). Given the high immunostaining intensity in fetal Leydig cells, this could imply that 17β-HSD-10 offers another mechanism whereby the Leydig cells are able to protect themselves from excessive estrogen influence in the pre- and immediately postnatal period, where estrogens may have marked and long-lasting disruptive effects on testis metabolism. Thus, 17β-HSD-10 could act in a way similar to that of estrogen sulfotransferase, whose genetic deletion in mice leads to severe impairment of spermatogenesis (32). In this context, this would suggest that 17β-HSD-10 itself might be yet another target for putative environmental disrupting agents, because a loss of its activity would potentiate the effects of any estrogens on the testis, as well as reducing local androgen activity, in the critical early phase of testis development. To date, there is no information on mammals with

a mutated 17β-HSD-10 or specifically reduced enzyme function. However, mutation of the homologous gene, *scully*, in Drosophila suggests an important role for this gene product in germ line formation and gonadal development (33).

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