AGE-RELATED CHANGES IN THE STEROID-PRODUCING CELLS OF RAT TESTIS

Darina Barbutska[1], Yveta Koeva[1], Mariana Bakalska[2], Nina Atanassova[2]

[1]Department of Anatomy, Histology and Embryology, Medical University of Plovdiv and [2]Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia

ABSTRACT

PURPOSE: Leydig cells are the main source of testicular hormones that control spermatogenesis, the male reproductive tract, and male secondary sexual characteristics. It is now well established that testosterone levels are reduced with ageing suggesting that there must be deficit in the steroidogenic capacity of ageing Leydig cells. In this respect, the present work aims at describing some structural and functional alterations in rat Leydig cells upon ageing.

MATERIAL AND METHODS: Light and electron microscopic observations and immunohistochemistry for 3β-hydroxysteroid dehydrogenase (3β-HSD) were used to identify some structural and functional features of the Leydig cells of ageing rats.

RESULTS: The routine histological analysis revealed that during aging (21 and 24 month- old rats) the Leydig cells undergo atrophic changes in size rather than reduction in their number and as consequence the interstitium of ageing testes appeared expanded. The immunoexpression of 3β-HSD as a marker of LC steroidogenic activity decreased after 18 months of age and the immunostaining considerably reduced in 24-month-old rats when compared to that in 3-month-old control ones. Ultrastructural study of Leydig cells in ageing rat testes revealed the presence of Leydig cells with intact morphology as well as Leydig cells with different degree of degeneration. An age-related progressive increase in the number of Leydig cells nuclei that exhibit apoptotic changes like chromatin fragmentation and compaction into dense masses leading to nuclear shrinkage was found out.

CONCLUSION: The results obtained suggest that effects of ageing on steroidogenesis are mainly due to structural and functional alterations in Leydig cells resulting in a decreased testosterone production.

Key words: *Leydig cells, 3β-hydroxysteroid dehydrogenase, ageing, electron microscopy, rats*

INTRODUCTION

Leydig cells (LCs) are the main source of testicular hormones that control spermatogenesis, the male reproductive tract and male secondary sexual characteristics. It is now well established that testosterone (T) levels are reduced with ageing, but LC number does not change, suggesting that there must be deficit in the steroidogenic capacity of aging LCs. Using old rats treated with ethane dimethanesulphonate (EDS) which destroys LCs, Chen *et al.* (5) have reported that newly-formed LCs restore high plasma T level in the old rats, indicating that the hypothalamic-pituitary axis in the old testis environment remain still intact. Indeed, it is unlikely that deficits of the hypothalamic-pituitary axis are

Address for correspondence:
Darina Barbutska, MD
Department of Anatomy, Histology and Embryology
Medical University of Plovdiv
15A Vassil Aprilov Str., 4000 Plovdiv, Bulgaria
e-mail: darinas5@abv.bg

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primarily responsible for age-related changes in steroidogenesis.

According to some authors (13), reduced aging LC ability to produce T might be caused by events occurring either outside these cells that impinge upon them, or occurring over time within LCs themselves involving accumulation of free oxygen radicals as byproduct of steroidogenesis. Ageing LCs are characterized by reduction of the number of luteinizing hormone (LH) receptor, cyclic adenosine monophosphate (cAMP) production (3,6), steroidogenic acute regulatory (StaR) protein, peripheral benzodiazepine receptor (PBR), cholesterol transport, and conversion of cholesterol to T by enzymes residing in the mitochondria and smooth endoplasmic reticulum (7,10,11).

The present investigation aims at describing some structural and functional alterations in rat LCs upon aging.

MATERIAL AND METHODS

Male Lewis rats at different ages (3,18,21 and 24 months) were used. Testicular fragments approximately 4-5 mm thick were fixed by immersion in Bouin's fluid for 24 hours, embedded in paraffin, and prepared for routine histological analysis (hematoxylin-eosin staining) and immunohistochemistry for visualization of 3β-hydroxysteroid dehydrogenase (3β-HSD). Primary rabbit polyclonal antibody against 3β-HSD (1:1000) kindly provided as a gift by Prof. I. Mason (Edinburgh University) was applied. Swine anti-rabbit biotinylated secondary antibody (E0353, DAKO) (1:500) was applied. Bound antibodies were visualized by incubating the sections with ABC complex/HRP reagent (K0355, DAKO) followed by colour development with DAB chromogene substrate (K3468, Liquid DAB+ kit, DAKO). For electron microscopy, testicular fragments were fixed in 2,5% glutaraldehyde, postfixed in 1% osmium tetraoxide and embedded in Durcopan. Ultrathin sections were observed on EM Opton 109.

RESULTS

By using light microscopic observation and routine histological analysis, LC atrophy with ageing (18-, 21- and 24 month-old rats) rather than reduction in LC number was established and as consequence, the interstitium of ageing testis appeared enlarged (Fig. 1 A, B). Ultrastructural study of LCs in rat testis revealed the presence of LCs with intact morphology as well as LCs with different degree of degeneration. We observed decreased smooth endoplasmic reticulum and mitochondria, fewer and smaller lipid inclusions and residual bodies. The immunoexpression of 3β-HSD as a marker of LC steroidogenic activity decreased after 18 months of age and the immunostaining considerably reduced in 24-month-old rats when compared to that in 3-month-old control ones (Fig. 2 A, B). During age-

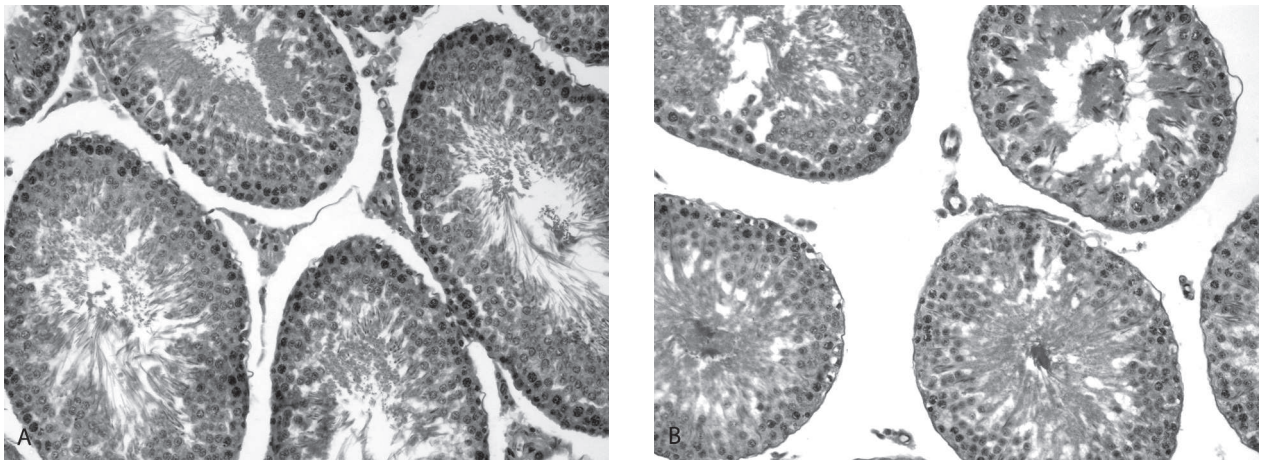

Fig. 1 A, B. Routine histological analysis (hematoxylin-eosin staining). In control testes (A) LCs are arranged in clusters or sheets of cells that surround the tubules and follow the course of blood vessels. Isolated forms in peritubular and perivascular positions are more often seen in 18- and 24-month-old rats, respectively (B). LCs undergo atrophic changes in size and as consequence the interstitium of aging testes appears augmented. Magn. x 200

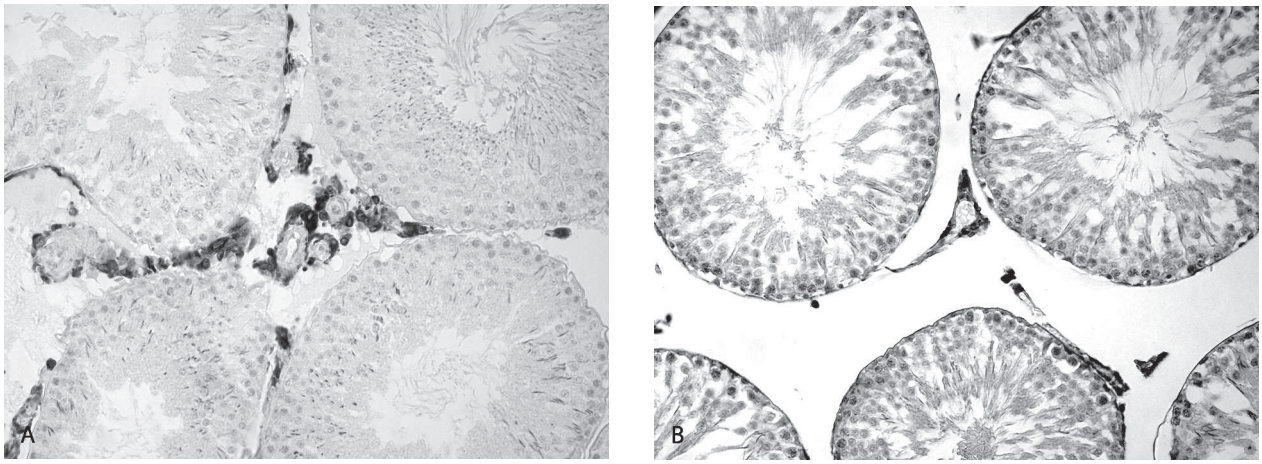

Fig. 2 A, B. Immunohistochemistry for 3β-HSD. 3-month-old control rats (A). 3-βHSD expression declines after 18 months of age and cytoplasm staining is strongly reduced at 24 months (B). Magn. x 200

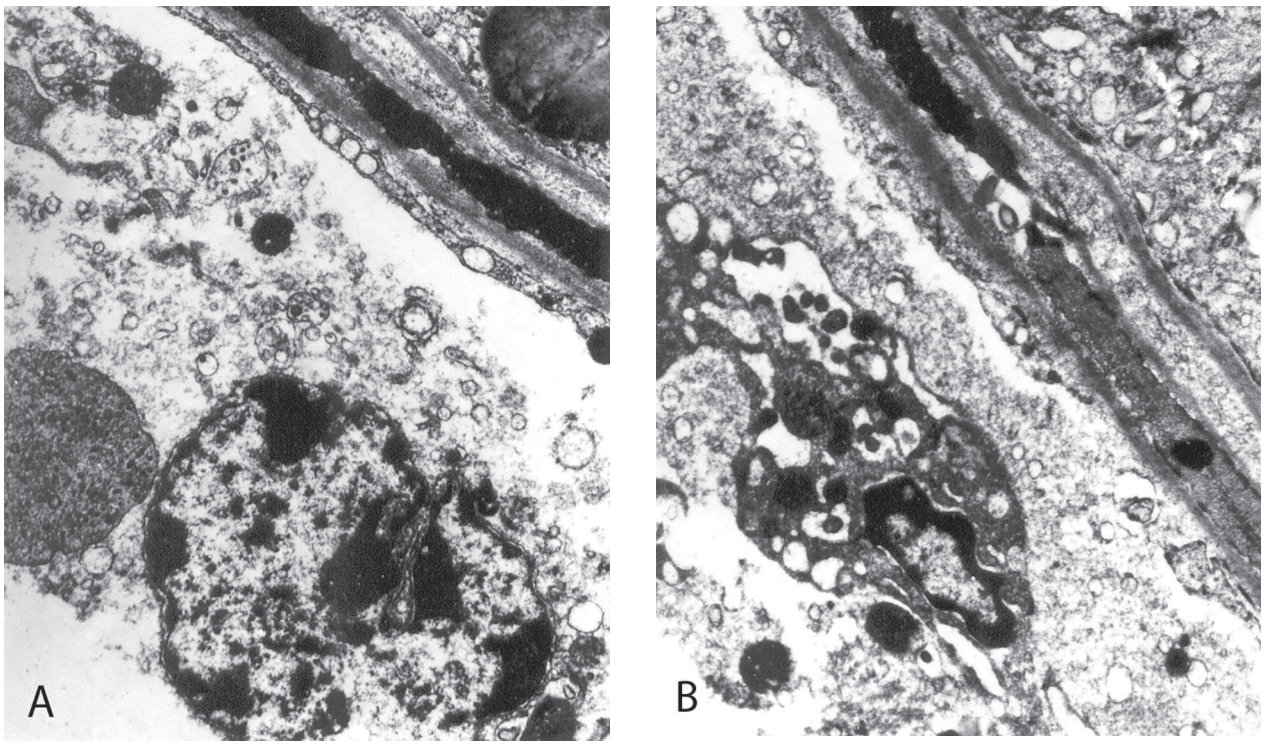

Fig. 3 A, B. Electron microscopic examination. Reduced smooth endoplasmic reticulum and mitochondria along with an accumulated lipid droplets and residual bodies in aging LCs (A). Apoptotic changes in LCs nuclei during aging consist in chromatin fragmentation and compaction into sharply defined dense masses leading to nuclear shrinkage and cellular volume diminution (B). Magn. x 4500

ing, apoptotic changes of LC nuclei such as chromatin fragmentation and compaction into dense masses inducing nuclear shrinkage were observed (Fig. 3 A, B).

DISCUSSION

Our results demonstrate LC atrophy during aging rather than LC number reduction. They are consistent with previous studies in rat testis regarding the effects of ageing on LC structure and

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functional activity. It has been reported that T levels reduce with ageing while LC number per testis does not change, suggesting that there must be a deficient steroidogenic capacity of individual LCs upon ageing (4,12).

The present findings of reduced 3β-HSD immunoexpression in aged rats are indicative of the suppressed steroidogenic LC activity and strongly correspond with previously published data about decreased serum T levels (3) and age-related reduction of the levels and activities of the key steroidogenic enzymes including 3β-HSD (1,9,10). The reduced size of LC population observed at light microscopy corresponds to the ultrastructural changes in the nucleus and cytoplasmic organelles. These alterations are indicative of decreased steroidogenic capacity of ageing LCs.

The observed ultrastructural alterations in LCs presenting with marked reduction in the major cytoplasmic organelles such as smooth endoplasmic reticulum and mitochondria could be considered the primary cause for impaired steroidogenesis during ageing. They are in accordance with previously reported data (2,8). Moreover, the progressive alterations in LC nuclei found out in the present study could be a possible sign of their increased apoptotic tendency during ageing.

CONCLUSION

The present results of ours suggest that age-related structural and functional alterations in LCs could be considered the primary cause for disturbed steroidogenesis and decreased T production during ageing.

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