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Mitochondrial Changes in Differentiating Rat Leydig Cells after EDS Treatment

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Mitochondria of steroid-producing cells are integrally involved with steroidogenesis. The presence of tubular cristae has been considered the hallmark of mitochondrial morphology in Leydig cells (LCs), as in other steroid-producing cells [16]. Treatment with ethane dimethanesulphonate (EDS, specific LC toxin) has become useful model for studying their development and function. The aim of the present study was to follow the mitochondrial changes in differentiating LCs after EDS in tandem with recovery of their steroidogenic function, indicated as NADH2-diaphorase activity. The newly formed LCs, appeared about 14 day post EDS treatment and corresponded to immature adult type LCs. Differentiation of immature LCs is associated with formation, modification and development of "organelle associations" responsible for steroid production. Our study provide data on detailed ultrastructural maturation of LCs involving diversity of changes and modification of steroidogenic organelles that was manifested by the recovery of Leydig cell enzyme activity and steroid producing function.

Key words: rat testis, Leydig cells, mitochondria, EDS, NADH2-diaphorase.

Introduction

In most species, testosterone is the major androgen secreted by Leydig cells (LCs) of the testis and its biosynthesis occurred in mitochondria that occupy substantial portion of the cell cytoplasm. Mitochondria of steroid-producing cells, whether gonadal or adrenal, have been distinguished from mitochondria of non-steroid-producing cells by the presence of predominantly tubular, rather than purely lamellar cristae [6, 16]. In steroid-producing cells of many species there is an intimate association of mitochondria with smooth endoplasmic reticulum [5]. The structural basis for maintaining such an association may be small filamentous attachments extending between these organelles [15].

In all species, the first and rate-limiting step in androgen biosynthesis is conversion of cholesterol to pregnenolone in mitochondria and it is catalyzed by the enzyme cytochrome P450 cholesterol side-chain cleavage (P450 scc) [10]. Subsequently, pregnenolone is metabolized by the enzymes 3 β -hydroxysteroid dehydrogenase (3β-HSD) and cytochrome P450 17α-hydroxylase in the SER to form the C19 steroids [11]. The

enzyme 3β-HSD catalyzed an essential step in the biosynthesis of all biologically active steroid hormones using NADPH as cofactor [9].

Ethane dimethanesulphonate (EDS) specificaly and temporarily eliminates LCs and hence this experimental model is an useful tool for studying development and differentiation of new Leydig cell population [4,18]. Ultrastructure of newly formed LCs after EDS administration is well described, but the changes of mitochondrial fine organization is not explored in detail, especially in relation to steroidogenic function.

In this respect, the aim of the present study was to follow the mitochondrial changes that occurred in differentiating LCs after EDS in tandem with recovery of their steroidogenic function, indicated as NADH2-diaphorase activity.

Materials and Methods

Mature Wistar male rats received EDS in a dose of 75 mg/kg body weight as previously described [3]. On day 1, 7, 14, 21, 35 post EDS administration, the testicular fragments were prepared for electron microscopy (EM). Ultrathin sections were observed in an Opton EM 109. For enzyme histochemistry, the other testis was frozen and enzyme reactions were performed on 7-μm frozen sections according to Nachlass et al. [11] for NADH2-diaphorase activity using β-nicotinamide adenine dinucleotide, reduced form, as a substrate. The sections were observed and documented using a Zeiss light microscope.

Results

Within 24 hours of EDS treatment Leydig apoptotic death occurred as we have been previously reported [3] and after 2-3 days all LCs were eliminated from the testis. The first morphological recognizable new LCs appeared about 14 days after EDS administration. Newly formed cells possessed characteristics of immature adult type LC in which dominated mitochondria with lamellar cristae (Fig.1 – *a*). Single cells weakly stained for NADH2-diaphorase (Fig. 2 – *a*) and 3β-HSD (not shown) could be found in the interstitial space of the testis.

On day 21 immature LCs possessed abundant Golgi apparatus, the complement of two types endoplasmic reticulum (rough and smooth) and mitochondria of both type, i.e. with lamellar and tubular cristae (Fig. 1 – *b*). In some LCs we observed involution and/or degeneration of mitochondria which looked swollen with electron pale matrix and some fragments from destroyed cristae (Fig. 1 – *c*). The apparent continuity of mitochondrial membrane with adjacent cisternae of smooth endoplasmic reticulum (SER) was also found. From day 21 and onward the enzyme reaction for NADH2-diaphorase became more pronounced (Fig. 2 – *b,c*).

At 35 day after EDS application in mature LCs the central apparatus for steroid production was fully formed and it was presented by SER, mitochondria with tubular cristae in electron dense matrix and peroxisomes. An intimate association of mitochondria with SER was also observed (Fig.1 – *d*).

Discussion

In the testis of human and other mammalian species two major Leydig cell populations appeared during testis development, namely a prenatal (fetal) and postnatal (adult) population [2]. The gradual turnover of adult LCs can be greatly accelerated by application of the experimental treatment with specific cytotoxic drug EDS. LCs were selectively

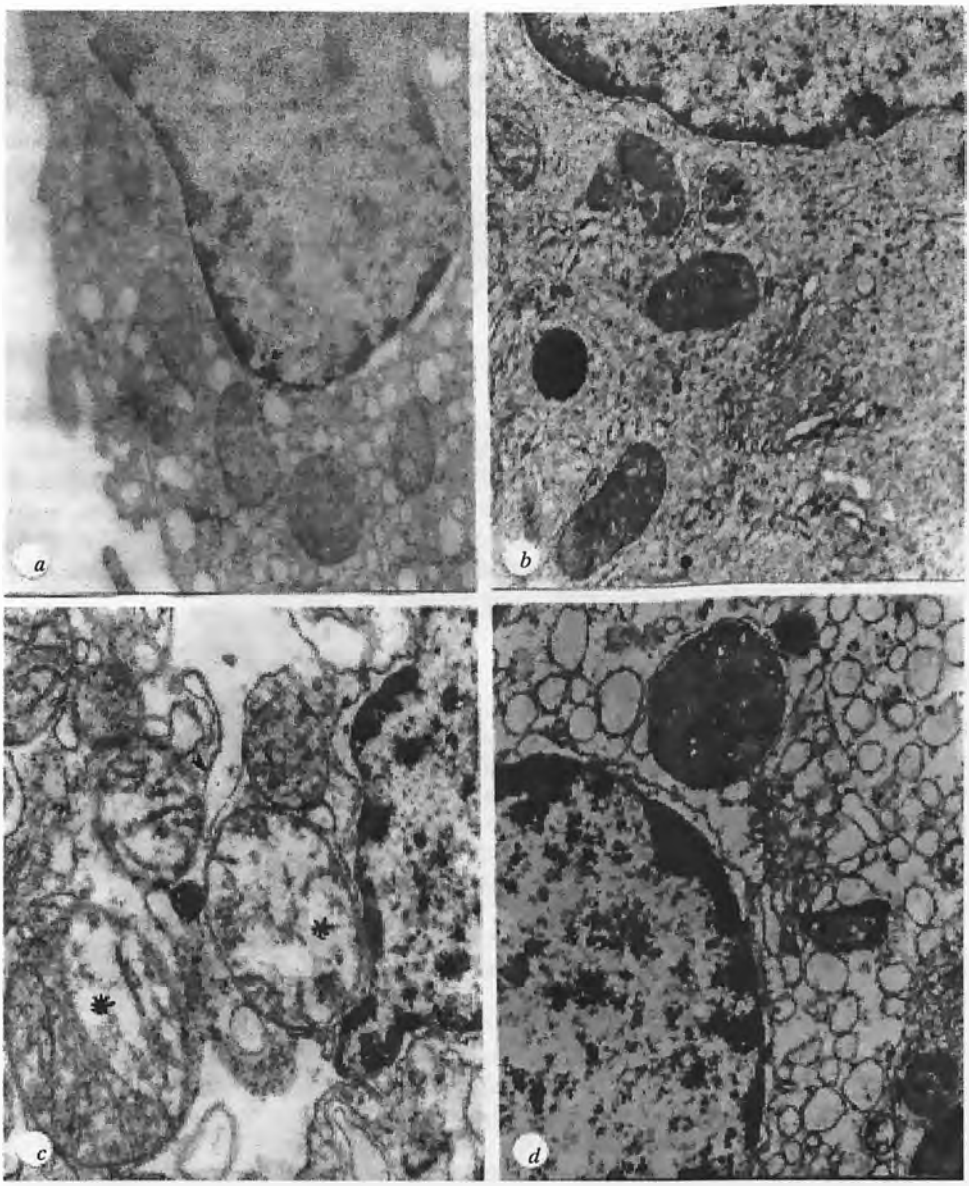

Fig.1. Electron micrographs of differentiating rat Leydig cells after EDS treatment:
a. On day 14 post EDS in cytoplasm of immature LCs mitochondria are relatively uniform with lamellar cristea (× 12 000)
b. On day 21 post EDS in immature LCs steroidogenic apparatus is developing and it is presented by smooth and regionalized rough endoplasmic reticulum , abundant Golgi elements and mitochondria with lamellar and tubular cristae (× 12 000)
c. On day 21 post EDS in some immature LCs there are mitochondria in process of involution and/or degradation (asterisks) as well as in reorganization. Note apparent continuity of mitochondrial membranes with the adjacent cisternae of SER (arrowhead) (× 20 000)
d. On day 35 post EDS cytoplasm of mature LCs exhibit fully developed steroidogenic apparatus with adundant SER and mitochondria with tubular cristae (× 20 000)

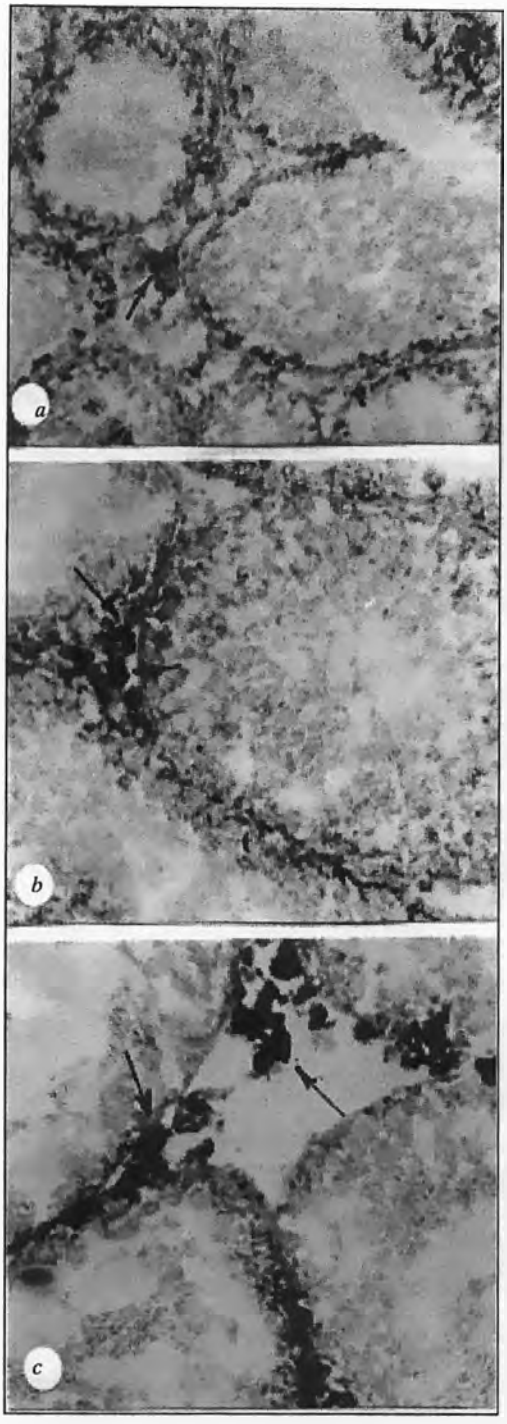

Fig.2. Histochemical visualization of NADH2-diaphorase activity in the differentiating Leydig cells (arrow) on frozen sections of the rat testis after EDS treatment:
a. On day 14 post EDS, in the intererstitial space of the testis, single newly formed LCs (arrow) showed moderate intensity of enzyme reaction (× 200)
b and c. On 21 and 35 day post EDS, respectively, enzyme reaction become more pronounced in increasing number of differentiating LCs (arrows) (× 400)

destroyed by EDS initiating a new wale of their development from stem cells, which remained unharmed [4, 8,]. Progenitor, immature and adult LCs have distinct sets of characteristics and may respond differently to the hormonal factors regulating their proliferation and functional differentiation [17,18]. Mitochondria, being the site of beta-oxidation, as well as the biosynthesis of pregnenolone from cholesterol, are very important organelles in steroid production [14]. Our data indicate that ultrastructural differentiation of newly formed LCs post EDS is associated with modification of mitochondria from type with lamelar to tubular cristae. Evolution of mitochondria and other steroidogenic organelles occurred in parallel with recovery of enzyme activity (NADH2-diaphorase and 3β-HSD) after EDS.

Intermediate forms (immature) LCs of postnatal development are described with "organelle associations" that include SER, lipid droplets and mitochondria [13]. Since side chain cleavage of cholesterol occurs in the mitochondrion and the remaining steroidogenic enzymes are located in the smooth endoplasmic reticulum (SER), there is an obvious physiological rationale for maintaining a close association between mitochondria and smooth endoplasmic reticulum [16]. During experimental renewal of Leydig cell population, we found close continuity of mitochondrial membrane with cisternae of SER. The apparent continuity of both membranes was reported by Prince [14]. Our finding indicated that intimate relationship between steroidogenic organelles is a part of evolution of steroidogenic apparatus not only throughout normal postnatal development, but also in adulthood during experimentally induced regeneration of LCs after EDS.

The dramatic morphological changes of mitochondria in some immature LCs observed in present study suggested an involution and/or degeneration of mitochondria in these cells. Similar mitochondrial changes, as noted in this study, have been described during LCs involution in the blue fox [1] and during the postnatal period in LCs of the monkey *Macaca fascicularis* [7].

In conclusion, our study provide data on detailed ultrastructural maturation of LCs involving diversity of changes and modification of steroidogenic organells that was manifested by the recovery of Leydig cell enzyme activity and steroid producing function.

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