Short Communication

Steroidogenic and Structural Differentiation of New Leydig Cell Population Following Exposure of Adult Rats to Ethane Dimethanesulphonate

(EDS / Leydig cell / ultrastructure / histochemistry)

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Abstract. EDS alkylating agent has been shown to selectively and temporarily kill LCs in adult rats. The first newly formed single LCs appeared at 14th day post ESD and showed detectable activity for 3β-HSD and NADH2-diaphorase, which became progressively stronger with time after treatment. The ultrastructural study revealed that the progenitor LCs differentiated into immature LCs within a week, and two weeks later they were transformed into mature LCs. Therefore, the restoration of new LC population after EDS treatment repeated the dynamics of normal LC development within a similar time range. The dynamics of enzyme activity correlated with structural differentiation of the new LC population.

The biosynthesis of androgens from cholesterol depends upon action of several enzymes located almost entirely in Leydig cells (LCs). The enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) catalyzes an essential step in the biosynthesis of all steroid hormones that requires the reduced form of nicotinamide adenine dinucleotide (NADH2) as a cofactor. This enzyme is the most active of the enzymes involved in testosterone biosynthesis (O'Shaughnessy and Murphy, 1991).

Ethane dimethanesulphonate (EDS) specifically and temporarily destroys Leydig cells, which results in a drop in testosterone levels and disturbance of pituitary-testicular hormonal axis reflected by gross elevation of serum gonadotropin levels (Bartlett et al., 1986; Kerr et al., 1987). This substance can, therefore, be used to study the role of Leydig cells in normal testicular function. Within two weeks of Leydig cell destruction by EDS, regeneration of new Leydig cell population has begun from precursor cells within the interstitium (Teerds, 1996; Morris et al., 1997; Kancheva et al., 2000). The morphology of the regenerating Leydig cells has been well described (Kerr et al., 1987; Hatier and Grignon, 1997), but their functional features during the same period are largely unclear. The Leydig cell precursors that differentiate into mature Leydig cells should also at the same time undergo functional differentiation. In this respect two questions arise: 1) when the steroidogenic enzyme activity could be first detected and 2) whether this activity shows a normal pattern of development during Leydig cell regeneration after EDS.

In this respect the design of the present study was to establish changes in steroidogenic enzyme activity in Leydig cells in relation to their structural differentiation during the period of Leydig cell renewal after EDS.

Material and Methods

Adult Wistar male rats received a single intraperitoneal injection of EDS at a dose of 75 mg/kg body weight dissolved in dimethylsulphoxide and water (1 : 3, v/v). Rats were killed on days 14, 21 and 30 after initial treatment (N = 4 per group). One testis was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and 1% osmium tetraoxide and embedded in Durcupan. Ultrathin sections were observed in an Opton electron microscope 109. For enzyme histochemistry, the other testis was frozen and enzyme reactions were performed with 7-μm frozen sections according to Levy et al. (1959) for visualization of 3β-HSD with substrate dehydroepiandrosterone and to

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Abbreviations: EDS – ethane dimethanesulphonate, HSD – hydroxysteroid dehydrogenase, NADH2 – reduced form of nicotinamide adenine dinucleotide, LC – Leydig cell, cyt P-450scc – cytochrome P450 cholesterol side chain cleavage.

Nachlass et al. (1958) 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.

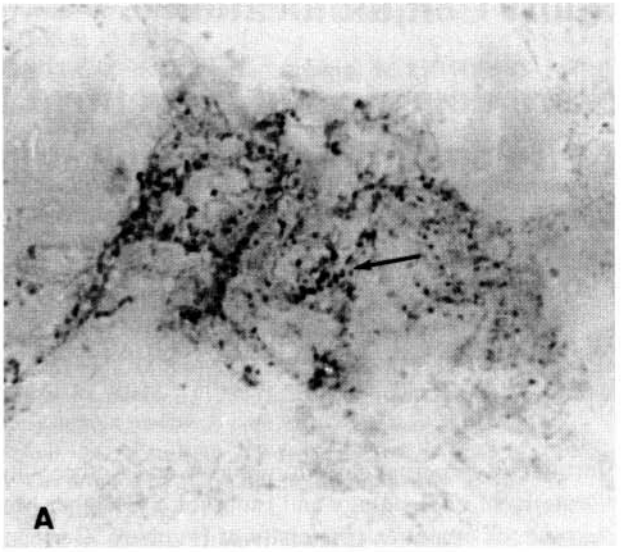
Results

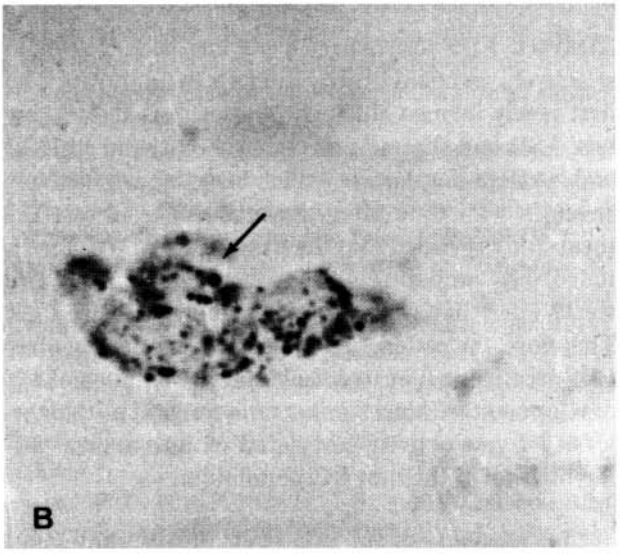
Our observations of control adult rat testis showed a strong activity in LCs for both enzymes, 3β-HSD and NADH2-diaphorase. The reaction product in LC cytoplasm was visualized as fine granules that were more abundant for NADH2-diaphorase in comparison to 3β-HSD (Fig. 1A and 1D). The 3β-HSD staining was specific for LCs whereas NADH2-diaphorase activity could be seen in the seminiferous tubules as well. The highly steroidogenic LCs were mature, adult-type, organized in clusters between the seminiferous tubules, or were in peritubular position.

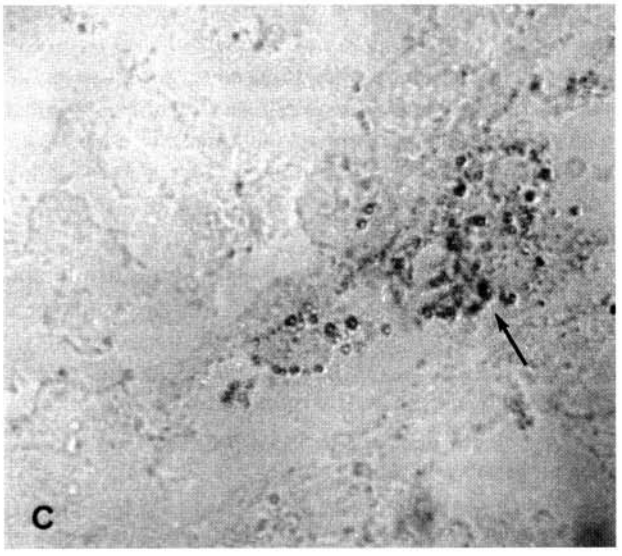
Two weeks after EDS treatment, single cells weakly stained for 3β-HSD or NADH2-diaphorase (not shown) could be found in the interstitial space of the testis. Electron-microscopical observation revealed the presence of a progenitor type of LCs with elongated spindle shape (Fig. 2A). They had little cytoplasm due to the virtual absence of the smooth endoplasmic reticulum (SER) and poor presence of the other cellular organelles in this stage. LC progenitors are intermediates in the LC lineage and they exist for only a brief interval (until at 21 days after EDS treatment), and after that they are transformed into immature LCs.

From day 21 to day 30 the intensity of histochemical staining for 3β-HSD increased in the cytoplasm of LCs (Fig. 1B and 1C) and reached the control pattern. The enzyme reaction for NADH2-diaphorase became more pronounced in the period investigated, but on day 30 it still remained weaker compared to the control (Fig. 1E and 1F). The changes in both enzyme activities coincided with numerous lipid droplets, mitochondria and appearance of SER – the specific ultrastructural characteristics for immature LCs (Fig. 2B).

On day 30 some of immature cells during their further differentiation obtained functional and morphological characteristics of mature adult-type LCs. The transition between immature and mature adult LCs was marked by a decline in cytoplasmic lipid droplets, abundance of SER and higher numbers of mitochondria with tubular cristae (Fig. 2C). An intimate association of mitochondria with SER was also observed.







Discussion

The EDS-treated rats have become a widely used model for studies on LC development, as well as for investigation of testicular function in the absence of LCs (Tena-Sempere, 1997).

The present study used this model to provide data on functional properties of regenerating LCs related to their structural maturation, which involves development of specific cellular organelles for steroidogenesis (smooth endoplasmic reticulum, tubular mitochondria, lipid droplets). In this regard we compared the histochemical data with electron microscopic observation. Our histochemical study demonstrated an increasing