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Morphometric and ultrastructural analysis of stage-specific effects of Sertoli and spermatogenic cells seen after short-term testosterone treatment in young adult rat testes

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Summary

The effects of testosterone treatment on spermatogenesis in the rat have been investigated by morphometric and structural analysis at the ultrastructural level in stages VII—IX. The aim has been to characterize the changes in Sertoli and spermatogenic cells to elucidate the mechanism of testosterone effects on spermatogenesis and to test the possibilities of developing male contraceptives. In stage VII, the morphometric parameters of volume and surface area in Sertoli cells (see abbreviations below): and the morphometric parameter of volume in the spermatogenic cells such as $V_{VPG,T}$, $V_{VPC,T}$, $V_{VrPT,T}$ and $V_{VelPT,T}$ decreased. In stage VIII, the respective values of Sertoli cells, VSN, and VSN/VSC decreased while SSJ increased, and the respective morphometric parameters in the spermatogenic cells, $V_{VPG,T}$, $V_{VPC,T}$, and $V_{VrPT,T}$ increased. In stage IX, in Sertoli cells VSC, VSN, VSN/VSC, and SSJ remained unchanged. In spermatogenic cells $V_{VPG,T}$, $V_{VPC,T}$, and $V_{VrPT,T}$ increased. Further, in all stages, a close apposition of mitochondria and rough endoplasmic reticulum in basolateral cytoplasm of Sertoli cells suggested active protein synthesis. In elongated spermatids in stage IX the microtubular manchette became disorganized. This disorganization and the unexpected shift after testosterone treatment from decrease in several morphometric parameters in stage

Abbreviations: VSC, absolute volume of an average single Sertoli cell; VSN, absolute volume of an average single Sertoli nucleus; SSJ, an average surface area of inter-Sertoli tight junctions in a single Sertoli cell; $V_{VPG,\ T}$, volume density of spermatogonia (PG) in the reference space of testes; $V_{VPC,\ T}$, volume density of spermatocyte (PC) in the reference space of testes; $V_{VrPT,\ T}$, volume density of round spermatid (rPT) in the reference space of testes; $V_{VelPT,\ T}$, volume density of the elongated spermatid (elPT) in the reference space of testes

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VIII to increases in stage IX cannot be explained by alterations in testosterone (T), LH, FSH, and their respective receptors. Therefore, still unknown regulatory factors in spermatogenesis are apparently involved in the developmental interactions between Sertoli and spermatogenic cells.

Introduction

The Sertoli and spermatogenic cells in testes constitute a vital morphological and physiological entity of tissue, which is regulated by the testicular-hypothalamic feedback loop. Removal of the hypophysis in rats (Russell and Clermont, 1977) causes specific changes in stages VII and VIII represented by a degeneration period in the pachytene spermatocyte, step 7 and 19 spermatids, apparently due to the lack of LH. Further, in early stage post hypophysectomy in rats, degeneration of preleptotene spermatocytes and abnormal manchette in elongated spermatids were found in testes, and it was suggested that these changes result from the lack of stimulation by the Sertoli cell (Ghosh et al., 1991). The principal action of testosterone (T) as proposed by O'Donnel et al. (1994) is to facilitate the maturation of round to elongated spermatids during spermiogenesis. However, a biphasic effect of androgen administration on Sertoli cell function in rats was recognized to depend on the dose (Weddington et al., 1976). Recently, it was confirmed that the administration of a correspondingly low dose of T (Weddington et al., 1976) for 1 week resulted in young adult rats in a rise of T in peripheral blood to a 7-fold concentration in comparison with that in control animals concomitant with markedly low levels of LH and FSH in the peripheral blood (Liu et al. 1997; Ichihara et al., 2001). This low LH level in peripheral blood was, according to morphometric analysis, accompanied by a reduction in the volume density of smooth-surfaced endoplasmic reticulum (sER) to 40% ($p<0.01$) in androgen-producing Leydig cells, and this apparently results in low testosterone and further in insufficient stimulation of Sertoli cells for the maintenance of spermatogenesis by T, since usually intratesticular T concentration is kept at approximately 60–100 times (Nishihara and Suzuki, 1980) or 50 times (Turner and Yamamoto, 1991) that in the peripheral blood. Recently Gendt et al. (2004) used Sertoli cell-selective AR knockout mice to show using morphometric analysis of their seminiferous tubular epithelium, that the numbers of spermatocytes, round spermatids, and elongated spermatids were reduced to 64%, 3% and 0%, respectively, while the same parameters in Sertoli cells were unchanged. They concluded that Sertoli cells regulate the development of spermatogenesis crucially through the process of meiosis of spermatocytes and subsequent process of developing spermatids by their intracellular androgen. The aim and rationale of the present study was to analyze quantitative and qualitative changes in Sertoli and spermatogenetic cells in rats treated with T for short period, focusing on changes at stages VII, VIII and IX as suggested by previous reports (Clermont and Harvey, 1965; Ghosh et al., 1991), of stages VII and VIII (O'Donnell et al., 1994), and stage VII (Sharpe et al., 1990). Concerning practical applications, the present treatment provides an animal model for male contraception in review of the previously observed marked changes in the local regulator Leydig cells (Ichihara et al., 2001).

Materials and methods

Experimental animals

Seven rats (Wistar strain) aged 12 weeks were separated into two experimental groups, four rats were used as control animals and three as an experimental group which were injected subcutaneously on their back with testosterone propionate at 1 mg daily for 7 days. The treatment was designed to suppress LH-dependent factors such as androgen from the Leydig cells and to cause lack of stimulation by Sertoli cells for spermatogenic cells.

Measurement of hormones

Blood samples were obtained from the inferior vena cava at the time when the testes were removed from the control and T-treated animals, and the plasma concentrations of T, LH and FSH were measured by radioimmunoassay methods (Nieschlag and Loriaux, 1972) (Fig. 1). T concentration was 3.13 ± 0.13 ng/ml in control rats, and 22.5 ± 0.62 ng/ml in T-treated rats. The increase to 7.18 times that of the control rats was statistically significant ($p<0.01$). LH decreased from 2.97 ± 0.15 ng/ml in