Testicular Denervation in Prepuberty Rat Inhibits Seminiferous Tubule Development and Spermatogenesis

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Abstract. In the adult rat, the superior spermatic nerve (SSN) and inferior spermatic nerve (ISN) are involved in regulating testosterone secretion and spermatogenesis, in addition to endocrine control mechanisms. However, these are currently few data on the demand nerve supply regulates testicular development and related mechanisms. The present study was thus designed to investigate the regulating effects of testicular nerve supply to testicular maturation, spermatogenesis and the involved mechanisms from prepuberty to adulthood in rats. We transected the SSNs and ISNs of rats on postnatal day (PD) 30 and then analyzed changes in testicular morphology and cauda epididymal sperm content, cell proliferation and apoptosis and primary spermatocyte meiosis on PD60 and PD90. The results demonstrated that testicular denervation significantly reduced testis mass, cauda epididymal sperm count and serum testosterone concentrations. Proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 immunohistochemistry staining proved that the denervation had no influence on the proliferation of spermatogonia and primary spermatocytes, but obviously promoted the apoptosis of round spermatids and Leydig cells. It is novel that denervation reduced the meiotic activation of zygotene and pachytene spermatocytes through the expression of synaptonemal complex protein 3 (SCP3)—a marker of meiosis. In addition, RT-PCR showed that testis denervation significantly decreased testis 3β-hydroxysteroid dehydrogenase 1 (3β-HSD1) and luteinizing hormone receptor (LHR) mRNA levels, but had no obvious influence on testosterone follicle stimulating hormone receptor (FSHR) mRNA expression. These results suggest that the testicular nerve supply plays an important role in supporting seminiferous tubule development and spermatogenesis from prepuberty to adulthood.

Key words: Apoptosis, Maldevelopment and spermatogenesis, Meiosis, Testicular denervation

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—Original Article—

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Abstract. In the adult rat, the superior spermatic nerve (SSN) and inferior spermatic nerve (ISN) are involved in regulating testosterone secretion and spermatogenesis, in addition to endocrine control mechanisms. However, these are currently few data on the demand nerve supply regulates testicular development and related mechanisms. The present study was thus designed to investigate the regulating effects of testicular nerve supply to testicular maturation, spermatogenesis and the involved mechanisms from prepuberty to adulthood in rats. We transected the SSNs and ISNs of rats on postnatal day (PD) 30 and then analyzed changes in testicular morphology and cauda epididymal sperm content, cell proliferation and apoptosis and primary spermatocyte meiosis on PD60 and PD90. The results demonstrated that testicular denervation significantly reduced testis mass, cauda epididymal sperm count and serum testosterone concentrations. Proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 immunohistochemistry staining proved that the denervation had no influence on the proliferation of spermatogonia and primary spermatocytes, but obviously promoted the apoptosis of round spermatids and Leydig cells. It is novel that denervation reduced the meiotic activation of zygotene and pachytene spermatocytes through the expression of synaptonemal complex protein 3 (SCP3)—a marker of meiosis. In addition, RT-PCR showed that testis denervation significantly decreased testis 3β-hydroxysteroid dehydrogenase 1 (3β-HSD1) and luteinizing hormone receptor (LHR) mRNA levels, but had no obvious influence on testosterone follicle stimulating hormone receptor (FSHR) mRNA expression. These results suggest that the testicular nerve supply plays an important role in supporting seminiferous tubule development and spermatogenesis from prepuberty to adulthood.

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marker of meiosis that is essential for spermatid formation [19]. We also measured the mRNA levels of luteinizing hormone receptor (LHR), follicle stimulating hormone receptor (FSHR) and 3β-hydroxysteroid dehydrogenase 1 (3β-HSD1) in the testes. Our aims were to elucidate the mechanisms involved in the regulation of testicular maturation and spermatogenesis by the SSN and ISN.

Materials and Methods

Animals and surgery
Sixty male Wistar rats obtained at PD23 were maintained in cages under a 12/12 h light/dark cycle at a controlled temperature (23 ± 2 C), with water and food provided ad libitum. All experimental animal use for this study was approved by the Chinese Association for Laboratory Animal Sciences. The animals were randomized into three groups: an intact control group (CON), a sham operation group (SHAM) and a group subjected to bilateral surgical removal of the SSN and ISN (S+I). Surgery was carried out one week after the rats arrived, as described previously [8, 11]. Briefly, rats were denervated under anesthesia with an intraperitoneal injection of sodium pentobarbital (40 mg/kg of body weight). Using a dissection microscope, the SSN and ISN were separated from the testicular artery and the inferior testicular ligament and excised along with portions of the nerves. Sham denervated rats were treated similarly, with separation but not excision of the nerves.

Serum and tissue collection
Blood samples were collected on PD60 and PD90 by cardiac puncture from each rat under sodium pentobarbital anesthesia as above. Serum was separated by centrifugation, frozen and stored at –20 C for testosterone assay. Then the rats were euthanized by an overdose of sodium pentobarbital. The right testes were removed, snap frozen and stored at –80 C for molecular analyses. The left testes were weighed, measured and then fixed in 4% paraformaldehyde (PFA) for histology and immunohistochemistry. Relative testis weight (absolute testis weight/body weight) and relative testis size (absolute testis anteroposterior diameter/body weight) [20] were used to estimate the effects of denervation on testicular development.

Testosterone concentration was assayed by radioimmunoassay (RIA) using commercially available kits (Northern Biotechnology, Beijing, China). The sensitivity was 2 pg/ml; the intra-assay coefficient of variation was <9.5%, and the interassay coefficient of variation was <10.3%.

Cauda epididymal sperm count
The left epididymis was isolated from each testis. The cauda region was removed, weighed, minced with ophthalmologic scissors and then homogenized for 10 min in 4.0 ml physiological saline solution [21, 22]. The homogenate was filtered through a nylon mesh, and then 1 ml filtrate was diluted with 4 ml saline solution containing 4% Trypan blue vital dye. From this solution, 20 μl aliquots were placed in a hemocytometer for counting the number of sperm/g of each cauda.

Morphometry
Five testicular histological sections per animal were stained with hematoxylin and eosin (H & E), totaling 100 seminiferous tubules/animal. The areas of the seminiferous epithelium and lumen were measured using Adobe Acrobat 7.0 Professional [23]. The following four categories classified according to the tubular area were used to evaluate the seminiferous tubules: a) reduced (19–51 × 10³ μm²), b) small (51–83 × 10³ μm²), c) medium (83–115 × 10³ μm²) and d) large (115–147 × 10³ μm²) [23, 24].

Immunohistochemistry (IHC)
Testes from PD60 and PD90 rats were fixed in 4% PFA in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) overnight at 4 C, dehydrated and embedded in paraffin wax. Five-micrometer-thick sections were cut and processed for proliferating cell nuclear antigen (PCNA) and caspase-3 IHC. Briefly, the sections were deparaffinized, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H₂O₂) in PBS for 30 min. Antigen retrieval was performed by microwaving the sections (3 × 4 min at medium power) in 0.01 M sodium citrate buffer (pH 6.0). The slides were then rinsed in PBS, blocked with 10% normal goat serum and incubated respectively with the primary antibodies PCNA (diluted 1:2000 in PBS, Sigma-Aldrich, St Louis, MO) and rabbit anti-cleaved caspase-3 monoclonal antibody (diluted 1:100; Cell Signaling Technology, Boston, MA, USA) overnight at 4 C. They were then rinsed and incubated with secondary antibodies, biotinylated goat anti-mouse and goat anti-rabbit (diluted 1:150 in PBS; Zymed, San Francisco, CA, USA), for 3 h at room temperature. Sections were washed in PBS and incubated with streptavidin-labeled peroxidase complex (diluted 1:150 in PBS; Zymed) for 3 h at room temperature, and the antibody was then visualized with 0.6 mg/ml 3,3′-diaminobenzidine tetrachloride (DAB, Sigma-Aldrich) dissolved in PBS, to which 0.03% H₂O₂ was added (brown staining); the sections were subsequently counterstained, blued, dehydrated and sealed with neutral balsam. Negative control sections were performed in a similar manner, except the primary antibody was substituted with PBS.

PCNA-labeled cells were identified by brown nuclear staining. The numbers of PCNA-positive cells and total cells (Sertoli cells, spermatogonia, spermatocytes and spermatids) per tubule were quantified in 10 tubules/animal [23]. Caspase-3-labeled cells were identified by brown nuclear, cytoplasmic and whole cell staining, as caspases translocate from the cytoplasm to the nucleus after activation [25].

Immunofluorescence
Synaptonemal complex spreads of spermatocytes were subjected to immunofluorescence staining as described previously [26]. The cross sections from the rat testes of the CON, SHAM and S+I groups on PD60 and PD90 were deparaffinized, subjected to antigen retrieval and incubated with the primary antibody, rabbit anti-SCP3 (diluted 1:500 in PBS; Abcam, 15093), overnight at 4 C in a moist chamber; the slides were then rinsed in PBS for 3 × 5 min and incubated for 2 h with the secondary antibody, biotinylated goat anti-rabbit (diluted 1:150 in PBS; Zymed), at room temperature. After rinsing the slides in PBS for 3 × 5 min, they were...
incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin (Southern Biotech, Birmingham, AL, USA) at a 1:50 dilution for 3 h in the dark. After rinsing, the slides were stained with Hoechst 33258 at a 1:50 dilution in the dark for 40 min. After a final rinsing in PBS, the slides were mounted with 75% glycerol in PBS. Observations were made on a fluorescence microscope (Leica LB30T, Germany) equipped with a filter for FITC (Leica, I3-513808). Images were captured using a digital camera (Leica DFC320).

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from the testes of the CON, SHAM and S+I groups at PD60 and PD90 using Trizol (Invitrogen, Carlsbad, CA, USA). Two micrograms of total RNA was reverse transcribed using 200 U of Superscript II Rnase H-Reverse Transcriptase (Gibco BRL, Bethesda, MD, USA) in a 25 μl reaction volume in the presence of 10 μM Oligo (dT), first strand buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl2), 0.01 M dithiothreitol and 10 μM each of dATP, dGTP, dCTP and dTTP. The RNA and Oligo dT mix were heated at 65 C for 5 min and then cooled to 4 C. The other reagents were added, and RT was performed at 42 C for 1 h.

PCR amplifications of 3β-HSD1, LHR, FSHR and GAPDH (a housekeeping gene) were carried out using primer pairs from published sources (Table 1). The 3β-HSD1, LHR and FSHR cDNAs were amplified by PCR for 35 cycles (94 C for 30 sec; the annealing temperatures were 55, 52 and 54 C, respectively, for 30 sec and then 72 C for 30 sec). GAPDH cDNAs were amplified by PCR for 30 cycles (94 C for 30 sec, 50 C for 30 sec and 72 C for 40 sec). The sizes of the PCR products were determined by comparison with a gene marker (100 bp DNA, Promega, Madison, WI, USA) run in parallel with RT-PCR products in 1.2% agarose gels containing ethidium bromide. The relative band intensity was quantified run in parallel with RT-PCR products in 1.2% agarose gels containing ethidium bromide. The relative band intensity was quantified.

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>Product size (bp)</th>
<th>Annealing temp. (C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-HSD1 F: ACTGCAGGAGGTCCAGGTCT</td>
<td>565</td>
<td>55</td>
<td>[27]</td>
</tr>
<tr>
<td>R: GCAAGTTAAACACAGAATACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LHR F: AGATGTATCCCCTGGAAAAAGGA</td>
<td>273</td>
<td>52</td>
<td>[28]</td>
</tr>
<tr>
<td>R: TACTCCCTTGGAAAGCATTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSHR F: TCTTCAGGGACTCTCTCGC</td>
<td>234</td>
<td>54</td>
<td>[29]</td>
</tr>
<tr>
<td>R: TCTTGAATCTCGGCTGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH F: AAACCCCATCACCATCTCCTCAG</td>
<td>361</td>
<td>54</td>
<td>[30]</td>
</tr>
<tr>
<td>R: AGGGGCCATCACAGTCTTCT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F: Forward. R: reverse.

Statistical analysis

All experiments were repeated at least three times, and the results are expressed as means ± SEM (n=10 per group). All data were analyzed using one-way analysis of variance (ANOVA) to determine the differences among the S+I, SHAM and CON groups using the SPSS software (ver. 10.0, SPSS, Chicago, IL, USA). Groups were considered significantly different if P<0.05.

Results

Effects of denervation on testicular development

As shown in Fig. 1, the relative testis weight (4.33 ± 0.33 g/kg) and relative testis anteroposterior diameter (8.96 ± 0.16 cm/kg) of the S+I group were significantly reduced (P<0.05) on PD60 compared with the controls (6.61 ± 0.14 g/kg and 10.93 ± 0.42 cm/kg respectively). On PD90, the relative testis weight (3.07 ± 0.53 g/kg) and anteroposterior diameter (4.91 ± 0.20 cm/kg) in the S+I group were significantly lower (P<0.01) than in the controls (4.97 ± 0.03 g/kg and 6.46 ± 0.03 cm/kg, respectively). There were no significant differences between the CON and SHAM groups on PD60 or PD90. These data demonstrate that SSN and ISN transection before puberty inhibited testicular development, and those effects persisted until sexual maturity and adulthood in the rat.

Denervation caused seminiferous tubule atrophy and decreased the cauda epididymal sperm count

Germ cells were organized in concentric layers in seminiferous tubules with a normal shape, and the tubular lumen was filled with mature spermatids in the CON and SHAM groups. However, in the S+I group, the seminiferous tubules were irregular and had disarranged epithelial layers and much fewer mature spermatids. In addition, many seminiferous tubules with exfoliated epithelia were observed in the S+I testes on PD 90 (Fig. 2A).

Denervation significantly reduced the seminiferous tubular areas. In the S+I group, the areas of 55% of the tubules on PD60 and 59% of the tubules on PD90 were found to have decreased to less than 51 ± 103 μm2, respectively. Normally, the areas of 58% of the tubules on PD60 and 77% of the tubules on PD90 were maintained at more than 83 ± 107 μm2 in the controls (Fig. 2B).

The numbers of spermatozoa per gram of cauda epididymis in the S+I group (7.21 ± 2.57 × 107/g on PD60 and 4.38 ± 1.40 × 108/g on PD90) were reduced significantly compared with the controls (22.39 ± 0.5 × 107/g on PD60 and 11.20 ± 0.47 × 108/g on PD90), respectively (P<0.01). There was no significant difference between the CON and SHAM groups (Fig. 2C). These results demonstrate that the testicular nerve supply plays an important role in
regulating seminiferous tubule development and spermatogenesis.

Effects of denervation on germ cell proliferation
To check the effects of testicular denervation on germ cell proliferation, we performed PCNA IHC and cell counting. PCNA positive cells were located in the contorted seminiferous tubules, which included spermatogonia and primary spermatocytes (Fig. 3). Although the distorted seminiferous tubules were small with abnormal morphology, the PCNA positive cells had similar distribution patterns in the CON, SHAM and S+I group animals on PD60 and PD90. The mean numbers of PCNA-labeled germ cells in the tubules of the S+I animals decreased significantly on PD60 (P<0.05), but showed no clear difference on PD90 compared with the CON groups (Table 2). However, the total numbers of cells per tubule in the S+I group were reduced significantly (P<0.01) on both PD60 and PD90 (Table 2). Thus, the denervation before puberty did not affect the proliferation of spermatogonia and primary spermatocytes, but inhibited the development of primary spermatocytes to spermatids.

Apoptosis of germ cells and Leydig cells induced by resection of the SSN and ISN
We evaluated the effects of testicular denervation on cell apoptosis using caspase-3 IHC. Caspase-3 positive immunostaining was observed in large numbers of Leydig cells and round spermatids in the S+I group, whereas very few positive cells were observed in the control group (Fig. 4). However, no caspase-3 positive staining was observed in Sertoli cells, spermatogonia or primary spermatocytes in any group on PD60 and PD90 (Fig. 4).

These results suggest that the SSN and ISN are involved in maintaining the survival of Leydig cells and spermatogenesis.

Denervation inhibits meiotic activation of primary spermatocytes
The immunofluorescence results showed that SCP3 colocalized with homologous sister chromatids during the zygotene and pachytene stages of meiosis. The spreads of SCP3 immunostaining showed a bouquet configuration at the zygotene stage and appeared as short rods at the pachytene stage in the CON group, whereas the expression level of SCP3 in the S+I group was significantly decreased on both PD60 and PD90 (Fig. 5). Thus, the meiotic activation of primary spermatocytes, which is essential for formation of spermatozoa, was obviously inhibited by testicular denervation in the prepubertal rats.

Effects of denervation on serum testosterone levels
Table 3 shows that the mean serum testosterone concentration in the S+I group was significantly lower than the control on both PD60 and PD90. However, there were no differences between the CON and SHAM groups. These results indicate that testicular denervation inhibits testosterone secretion.

Effects of denervation on 3β-HSD1, LHR and FSHR mRNA expression levels
The RT-PCR results (Fig. 6) showed that SSN and ISN deprivation significantly decreased the LHR mRNA levels on PD60 and the 3β-HSD1 and LHR mRNA levels on PD90, respectively (P<0.05), but there were no obvious differences in the FSHR
mRNA levels among the S+I, CON and SHAM groups on PD60 and PD90. These results indicate that testicular denervation affects the expression of 3β-HSD1 and LHR mRNA in Leydig cells.

Discussion

Testicular denervation at prepuberty markedly affected the development of seminiferous tubules and spermatogenesis by inducing apoptosis in germ cells and Leydig cells, thereby inhibiting the meiotic activation of zygotene and pachytene spermatocytes during maturation. Thus, testicular innervation significantly modulates testicular development in the prepubertal rat.

Surgical removal of the SSN and ISN in these prepubertal rats induced reductions in testicular mass and in serum testosterone to below physiological levels at sexual maturity and adulthood. This is consistent with reports that testicular denervation caused abnormal development of the testis and a significant reduction in serum testosterone [11, 31, 32]. However, bilateral denervation in the adult rat has no acute or chronic effects on testicular weight, spermatogenesis or basal plasma testosterone level [2]. This supports our hypothesis that the testicular nerve supply is more important in regulating testicular development in the prepubertal rat than in adults.

SSN and ISN transection clearly affected the development of seminiferous tubules. Disarranged epithelial layers and reductions in tubular area were observed in the denervated testes. Without a nervous supply, the testicular blood vessels do not change blood flow in response to environment stresses [2, 33]. Thus, the testes are unable to adapt to the changes in air temperature that can inhibit the development of the seminiferous tubules [34]. The nervous
supply has chronic trophic effects on testicular development [12]. This was confirmed in our experiment by the fact that the weights and tubular areas of denervated testes were lower on PD90 than on PD60. This abnormal development of seminiferous tubules is presumed to be the main cause of inhibiting spermatogenesis [23, 24]. Thus, there were few mature spermatids in the seminiferous tubule lumen and a significant reduction in the cauda epididymal sperm count in the rats with denervated testes. Although deprivation of the SSN and ISN reduced the number of cauda epididymal sperm significantly, fertility and embryo numbers after mating with sex matured female rats were not significantly reduced (data not shown).

Normally, from the prepubertal to adult stages of development, the Leydig cell number increases by a cascade of differentiation, and the adult population is established by the end of puberty [18]. This enhances androgen production, which is essential for normal sexual behavior [35–36] and spermatogenesis [37]. However, the present results show that testicular denervation enhanced Leydig cells apoptosis. This reduced the total Leydig cell number, leading to a decline in serum testosterone and reduced cauda epididymal sperm numbers. These factors inhibited testicular growth and spermatogenesis and influenced the development of seminiferous tubules. This is supported by recent reports that testicular denervation in adult rats induced apoptosis in germ cells and Leydig cells [13, 14, 38].

We analyzed germ cell proliferation based on PCNA expression, an index of proliferative activity in the rat testis [39–41]. Notably, SSN and ISN transection did not inhibit the proliferation of spermatogonia or primary spermatocytes, although the contorted seminiferous tubules were small and had an abnormal morphology as described above. However, the total cell number per tubule was reduced significantly. These data suggest for the first time that proliferation of spermatogonia and primary spermatocytes from sexual maturity to adulthood is independent of testicular innervation, but that testicular innervation supports the development of primary spermatocytes to spermatids.

In addition, we found that transection of the SSN and ISN inhibi-
Hu et al. [376] cited the meiotic activity of zygotene and pachytene spermatocytes. During the zygotene and pachytene stages (synapsis), two parallel lateral elements become connected along their entire lengths by transverse filaments, and a central element of the SC is formed [42]. After synopsis, the homologous chromosomes separate, and the SC disassembles during diplotene and diakinesis [43].

Table 2. PCNA-positive cells and total cells (Sertoli cells, spermatogonia, spermatocytes and spermatids) per tubule of animals from the CON, SHAM and S+I groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCNA-labeled cells/tubule</th>
<th>Total cells/tubule</th>
<th>Percentage of PCNA-labeled cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (PD60)</td>
<td>86.5 ± 6.5</td>
<td>329.5 ± 23.6</td>
<td>26.4 ± 1.5</td>
</tr>
<tr>
<td>SHAM (PD60)</td>
<td>74.3 ± 7.3</td>
<td>297.3 ± 7.3</td>
<td>24.9 ± 1.9</td>
</tr>
<tr>
<td>S+I (PD60)</td>
<td>56.5 ± 6.5*</td>
<td>174.0 ± 21.3**</td>
<td>32.6 ± 1.8*</td>
</tr>
<tr>
<td>CON (PD90)</td>
<td>72.7 ± 7.6</td>
<td>278.0 ± 13.5</td>
<td>26.3 ± 2.8</td>
</tr>
<tr>
<td>SHAM (PD90)</td>
<td>72.7 ± 6.2</td>
<td>269.0 ± 5.1</td>
<td>21.8 ± 2.1</td>
</tr>
<tr>
<td>S+I (PD90)</td>
<td>42.5 ± 4.8</td>
<td>105.8 ± 14.4**</td>
<td>41.2 ± 3.4*</td>
</tr>
</tbody>
</table>

Asterisks (*, P<0.05; **, P<0.01) indicate statistical significance between the S+I and CON and SHAM groups.

Table 3. Mean serum testosterone concentrations in the CON, SHAM and S+I groups on PD60 and PD90

<table>
<thead>
<tr>
<th>Serum testosterone on PD60 (ng/ml)</th>
<th>Serum testosterone on PD90 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>SHAM</td>
</tr>
<tr>
<td>1.93 ± 0.32</td>
<td>2.41 ± 0.35</td>
</tr>
<tr>
<td>5.87 ± 1.08</td>
<td>7.62 ± 0.71</td>
</tr>
</tbody>
</table>

Asterisks (*) indicate statistical significance (P<0.05) between the S+I and CON and SHAM groups.

Fig. 6. Expression levels of 3β-HSD1, LHR and FSHR mRNAs in the S+I, SHAM and CON groups on PD60 and PD90. Asterisks (*) indicate statistical significance (P<0.05) between the S+I and CON and SHAM groups.
results, there was a clear inhibition of SCP3 spreads at the zygotene and pachytene stages in the denervated testes, whereas the replication of DNA in spermatogonia and primary spermatocytes was normal according to the expression of PCNA. These results are in agreement with a report that spermatogonial cells and preleptotene/leptotene spermatocytes were not affected in SCP3-deficient mice [19]. However, there was massive germ cell apoptosis in the denervated rat testes because the chromosomes did not undergo synapsis. This has also been observed in SCP3-deficient mice [19]. Thus, testicular innervation supports meiotic and postmeiotic germ cell development, although the related molecular mechanisms need to be elucidated.

It has been well documented that the development and functions of the testis are regulated by pituitary gonadotropin, FSH and LH. The LH receptor, localized in the Leydig cells, is involved in the regulation of steroidogenesis [37] and Leydig cell development [44]. FSH maintains the normal process of spermatogenesis through the FSHR in Sertoli cells [45, 46]. Therefore, we analyzed the effects of denervation on LHR and FSHR mRNA expression levels. The LHR mRNA level decreased significantly, but there was no change in FSHR mRNA. This is consistent with a previous report that bilateral nerve resection decreased testicular LHR expression [11]. One possible reason for this is that denervation at prepuberty prevents Leydig cell maturation. FSHR mRNA expression was not affected by denervation and spermatogenesis was not affected in FSH receptor knockout mice [47], although others have reported that FSH partially supports spermatocyte maturation in adult rats [25, 48]. The reduction of 3β-HSD1 in the present study is possibly connected with the massive Leydig cell apoptosis and LH regulates 3β-HSD1 and LHR mRNA expression in Leydig cells.

Acknowledgments

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References