The autonomic innervation of the testicular parenchyma: a rat model

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Summary

The specific roles and direct involvements of autonomic innervations on the spermatogenic process are poorly understood. The aim of this study was to investigate stereologically the relative importance of sympathetic innervations in testicular parenchyma rats in chemically sympathectomized with guanethidine. Treated animals (n=10) were injected intraperitoneally with guanethidine at doses of 10mg/kg/day for 15 days while control animals (n= 5) received an equivalent volume of saline. After routine histological procedures, 5µm thick sections of the testes were selected for examination. Organ volumes were estimated using the Cavalieri Principle of volume measurement by means of consecutive serial sections, using "J Images" software in a computer. At least 10 seminiferous tubules were selected randomly and measured per cross section for evaluation of epithelial heights, luminal diameter and total seminiferous tubule diameter. Testicular volumes and seminiferous tubule measurements of treated animals were found to be affected by the chemical sympathectomy with guanethidine with a a statistically significant difference between experimental and control group ($p<0.01$). Our findings indicate that chemical sympathectomy with-short term low dose guanethidine might display morphometric changes in the rat testis which indicate the presence of autonomic innervation of its parenchyma.

Introduction

The autonomic nervous supply to the internal genital organs of males are predominantly composed of short excitatory adrenergic fibres. Autonomic nerves using regulatory peptides as neurotransmitters and modulators have been reported to be present in mammalian male genitalia. Morphological studies have shown that the testicular autonomic innervations of most mammals seem to be restricted to the capsule and superficial blood vessels, thus strongly suggesting a role in the control of blood flow and temperature *(Karaguzel, 1995; Karnak et al, 1996; Kempinas et al, 1988).*

The specific roles and direct involvement of autonomic innervations on the spermatogenic process has not been fully established. The investigation of the testicular parenchymal structure after sympathectomy suggests the presence of its autonomic innervation. Due to the anatomy and physiology of

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the sympathetic nervous system (SNS), selective methods of SNS impairment and destruction are available *(Picklo,1997)*. Sympathectomy refers to the selective destruction of noradrenergic postganglionic sympathetic neurones and has been accomplished using a variety of chemical and surgical methods *(Demas & Bartness, 2000; Picklo,1997)*. The changes in the testes following chemical and surgical sympathectomy were reported in previous studies. The results, however, were different from each other.

No degenerative changes were observed in the germinal epithelium of rats by testicular denervation with extensive surgical pelvic sympathectomy *(Back, 1931)*. On the contrary, surgical and chemical testicular denervation caused degeneration of germ cells and arrest of the spermatic process in rats *(Nagai et al, 1982)* as well as in cats and guinea pigs *(Clark, 1933; King & Longworth, 1940; Shirai et al, 1965; Nagai et al, 1982)*, dogs *(Kuntz, 1919)*, and rabbits *(Hodson, 1965)*. Recently, studies with rats have shown that the autonomic nerves regulate the biosynthesis and secretion of testosterone by the Leydig cells. Sympathectomy causes a significant decrease in plasma and intratesticular testosterone levels as well as in androgen activity resulting in a lower degree of testicular maturation *(Lamano-Carvalho et al, 1996; Rosa e Silva et al, 1995)*. It has been proposed that the autonomic nervous system may be directly involved in the control of spermatogenic activity of the adult testis *(Shirai et al, 1965)*. However, in rats, the spermatogenic process seems to be unaffected by chemical sympathectomy *(Lamano-Carvalho et al, 1993)*.

There is a paucity of published reports regarding parenchymal effects and virtually no reports exist on the volumetric changes in the testes of sympathectomized rats. The aim of the present study was to investigate the presence of autonomic innervation of testicular parenchyma. For this, we used a chemical sympathectomy model to detect possible volumetric and structural changes.

Materials and Methods

Animals

Adult male Spraque Dawley Albino rats (about 187±29g body weight) were used. Rats were maintained under dark/light (14 d, 10 l) conditions and were provided with standard pellet chow and tap water ad libitum throughout the experiment.

Chemical sympathectomy procedures

Some of the animals (n= 10) were injected intraperitoneally with guanethidine (CIBA) at 10mg/kg/day for 15 days while control animals (n= 5) received equal an volume of saline *(Evans et al, 1972, Lamano-Carvalho et al, 1986)*. This procedure can selectively abolish the noradrenergic nerves containing neuropeptide Y (NPY) in the internal male genitalia *(Lamano-Carvalho et al, 1993).*

Histology

On the last day, the animals were sacrified and the left testes were removed and divided into two equal portions from the equatorial plane. Then, they were fixed in Bouin solution for 24 hours. After routine histological procedures, consecutive 5µm thick sections were cut at 600 µm intervals.

Stereology

The first section studied was selected randomly from a series of samples according to the Cavalieri Principle of "systematic random samples". About 10±3 cross sections were obtained from each half testis. Sections were routinely stained with hematoxylin and eosin. Images were obtained by computer-assisted microscopy using a Nikon SMZ 800 stereomicroscope under appropriate magnification. Images were captured with a CCD video camera (Hitachi-Japan) to a attached computer (PowerMac 7500) via a video-capture card and stored as TIF or JPG images. Testicular areas were analyzed using a software package *(J Images 1.27z program, public version of National Institutes of Health-USA)*. For conventional area estimation, the outer border of each testis was manually traced by the investigator

using a computer mouse. This procedure was repeated by two different persons at different times. The corresponding area per testis was then calculated. Calibration was set with an objective micrometer slide.

Organ volumes can be estimated using the Cavalieri Principle of volume measurement by means of consecutive serial section. Volume estimates were performed using the following formula (modified from Cavalieri) *(Gundersen et al, 1988; Howard & Reed, 1988).*

Volume = slice number x thickness x mean area size x 2 (half testis used).

Seminiferous tubules measurements: at least 10 seminiferous tubules, at stages IX and X of the germinal epithelium cycle *(Wing & Christensen, 1982)*, were selected randomly and measured per cross section. For this, the largest tubular and luminal diameters of the tubules were measured (in micrometers μm). Germinal epithelial (in μm) were heights-as follows. *(Canan et al, 2002; Malas et al, 2001; Sala et al, 1980):*

Germinal epithelium height = (Total diameter of tubule – Total diameter of lumen) / 2

Statistics

To ensure interobserver reliability, Kappa statistics were used *(Fleis, 1981)*. Kappa (κ) values higher than 0.75 may be regarded as excellent interobserver agreement and those below 0.40 as poor agreement. For statistical analysis: firstly; the groups were investigated by one-sample Kolmogorow-Smirnow test. P values higher than 0.05 indicate normal distribution of the data. The parametric Student-t (independent) test was preferred for statistical comparison of the control and treatment groups *(Hayran & Ozdemir, 1995)*. Data were expressed as mean ±SD (standard deviation) and $p < 0.05$ represents a significant difference.

Results

For all measurements interobserver reliability showed an excellent agreement (κ=0.85). All of the parameters examined were affected from lower dose sympathectomy with guanethidine. Significant alterations were found in both dimensional and volume measurements of treated animals. A de-

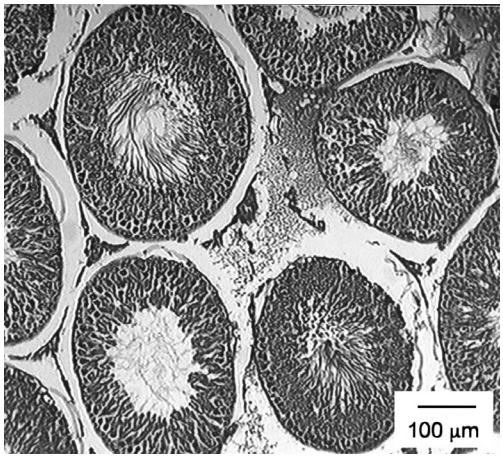

Figure 1. Cross sectional views of seminipherous tubules from a control animal (H&E)

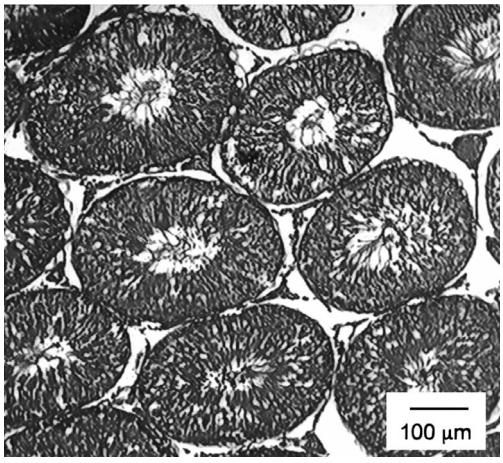

Figure 2. Cross sectional views of seminipherous tubules from a treated animal (H&E)

crease was observed in germinal epithelial height, luminal diameter and total seminiferous tubule diameter of sympathectomized rats (Figure 1, Figure 2). Similarly, a decrease was found in the volume of the testis of guanethidine-denervated rats ($p < 0.05$). These results are summarized in Table 1 and Figure 3 as dimensional changes and in Table 2 and Figure 4 as volume changes.

Discussion

The autonomic nervous system innervates the male reproductive tract by means of adrenergic, choliner-

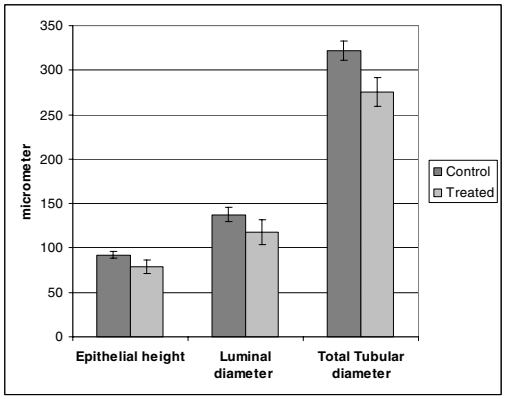

Figure 3. Seminiferous tubule measurements (µm) in treated (n= 10) and non-treated (n= 5) rats The dark bars represent measurements in control animals, and the light bars represent measurements in the treated animals with guanethidine. Data are plotted as means ±SD.

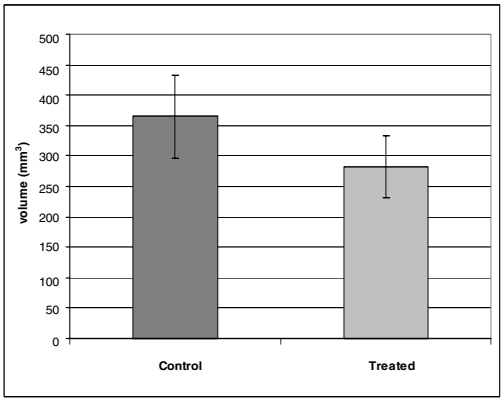

Figure 4. Testicular volume (mm3) in control and treated rats. The dark bars represent volumes in control animals and the light bars in the treated animals. Data are plotted as means ±SD.

		Mean	SD	P value (student –T)
Epithelial height (µm)	***Control***	92.21	4.114	0.00158*
	Treated	7 8.695	7.257	
Luminal diameter (µm)	***Control***	137.730	8.146	0.00000539*
	Treated	117.790	13.778	
Total tubular diameter (µm)	***Control***	322.150	10.797	0.0059**
	Treated	275.181	15.974	

Table 1. Effects of chemically sympathectomy with guanethidine in the germinal epithelium of rat testes: epithelial height, luminal and tubular diameter (n=10 in treated and n=5 in nontreated group), Mean (±SD) SD: Standard deviation, * P< 0,001, ** P< 0,01.

		Mean	SD	P value (student –T)
Volume (mm^3)	*Control*	364.782	67.70075	0.018677935*
	Treated	281.8766	50.48967	

Table 2. Effects of chemically sympathectomy with guanethidine in the volumes of rat testes: (n=10 in treated and n=5 in nontreated group), Mean (±SD) * P< 0,05.

gic, and nonadrenergic-noncholinergic (NANC) systems. The present study indicated the autonomic innervation of the testicular parenchyma in rats. Our morphometric examination of the rat testis showed significant volume and dimensional changes characterized by decreased epithelial heights, tubular and luminal diameter.

Early studies reported the absence of autonomic innervation of the testicular parenchyma in rats *(Dayan, 1970; Norberg et al, 1967; Risley & Screpetos, 1964)*. The seminiferous tubule epithelium is covered with peritubular tissue which contains some cells with the characteristics of smooth muscle cells, myoid cells *(Leeson et al, 1988)*. Since myoid cells form a layer in most rodents, it suggests that autonomic nerve fibres to the rat testis should be present. Indeed, the presence of the noradrenergic and NPY-immunoreactive nerve fibres to the rat testis was demonstrated *(Lamano-Carvalho et al, 1986)*. This innervation effect the vasoactivity within the testis *(Kumazawa et al 1987; Lamano-Carvalho et al, 1986)*. It was suggested that its role is in the control of blood flow and temperature *(Lamano-Carvalho et al, 1993)*. The present work further studied the effect of chemical sympathectomy on the rat testis. While the male accessory sex glands have been shown to be innervated by short adrenergic neurons located adjacent to the target organs *(Sjöstrand, 1965)*, the motor nerve supply to the testis is derived from long postganglionic fibres from the superior spermatic nerves or spermatic plexus *(Hodson, 1970)*. Morphometric studies showed that guanethidine with lower dosages (5-10 mg/kg/day) damages short adrenergic neurons earlier *(Lamano-Carvalho et al, 1993)* and the vas deferens of sympathectomized rats displayed marked dimensional changes characterized by an increased luminal area in contrast to a decrease in muscle layer area and epithelial height *(Aydınlıoglu et al, 2002; Kempinas et al, 1998; Lamano-Carvalho et al, 1993)*. However, chronic treatment of adult rats with low dosages of guanethidine also causes minor effects on adrenergic neurons innervating the testis, i.e. long postganglionic noradrenergic neurons *(Gannon et al, 1971; Evans et al, 1972)*. While widespread sympathectomy involves the degeneration of over 95% of peripheral noradrenergic neurons (short and long) *(Kempinas et al 1988)*, however, higher doses (25-30 mg/kg/day) of guanethidine are needed to destroy the testicular sympathetic innervation *(Burnstock et al, 1971; Evans et al 1972)*. Lamano-Carvalho et al (1993*)* reported that the spermatogenic activity of the testis was unaffected by guanethidine treatment with low dose and 30 days. Our study with low dosages and short term (10mg/kg, but 15 days) indicated the signs of impairment of the testes of denervated animals, namely germinal epithelium heights, tubular and luminal diameters changed. As stated above, low doses may affect the adrenergic neurons innervating the testis. Different findings in the present study might be explained by the shorter duration of the treatment. Alternatively, it might be due to the different quantitative technique we used in our study. In this study, we also investigated the effect of the guanethidine on the volume of the testis by using stereological methods. As with the dimensional changes, a decrease was found in the volume of the testes of sympathectomized rats. According to our knowledge, this is the first report of an alteration in

testis volume of chemically sympathectomized rats. In conclusion, chemical sympathectomy with low dose guanethidine over a short period displayed volume and dimensional changes on the rat testis which might indicate the presence of the autonomic innervation of testicular parenchyma. However, our observations should be confirmed with further studies.

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