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Effects of Chronic Stress on Penile Corpus Cavernosum of Rats

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ABSTRACT: The objective of this study was to investigate structural changes in the penile corpus cavernosum of prepubertal chronically stressed rats. Eight Wistar rats were assigned into the stress group (SG) and were submitted to 2 hours of tube restraint daily, from the fourth to the ninth week of life. Another 7 rats were used as the control group (CG). All animals were weighed weekly. At day 64, animals were sacrificed by anesthetic overdose, blood was collected for testosterone concentration by radioimmunoassay, and penis and adrenal were collected. Adrenal mass index and testosterone serum levels were used to assess the efficacy of the stress stimulus. The surface density of connective tissue and smooth muscle fibers of corpus cavernosum were measured on Masson trichromic–stained slices. Picrosirius red–stained slices were assessed under polarized light for different types of collagen. The Student's *t* test was applied for mean comparisons, with $P < .05$ considered significant. Testosterone serum concentrations decreased and adrenal mass index increased, confirming the effectiveness of the stress protocol. Smooth muscle fibers of corpus cavernosum decreased from 14.07% (CG) to 8.98% (SG) ($P = .02$), and connective tissue increased from 53.66% (CG) to 64.47% (SG) ($P = .01$). Also, there was a higher level of type I collagen in the SG animals compared with the CG. Stress stimuli induced structural changes in the corpus cavernosum of rats suggestive of penile fibrosis, which may play a role in erection dysfunction.

Key words: Androgen, penis, extracellular matrix, erectile dysfunction, morphology.

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Erectile dysfunction (ED) is the most common sexual complaint of men presenting to their physicians, with a worldwide prevalence between 10% and 20% (Albersen et al, 2011). Recognized risk factors for ED are diabetes, hypertension, cardiovascular diseases, smoking, and obesity, all of which are age related (Kubin et al, 2003).

Although ED has been extensively studied in older men, there are few published data regarding ED in young individuals (Tal et al, 2009). In a large sample accessed by telephone in France, ED was reported by 9% and 12% of men aged 18 to 19 and 20 to 24 years, respectively (Béjin, 1999). The underlying etiology and risk factors associated with juvenile ED have not been previously studied, but the origin of ED has been reported to be primarily psychogenic in at least 52% of teenaged patients (Tal et al, 2009).

Much of the research focusing on the risk factors for ED does not adequately consider the psychosocial variables that can contribute to this disorder, although there is evidence that stress and anxiety are highly associated with ED (Hunt and McHale, 2007). It is well known that chronically stressed male rats have decreased serum testosterone levels and impaired sexual behavior (Sato et al, 1996; Retana-Márquez et al, 2003). However, no evidence of structural changes on penile cavernous tissue has been verified.

Thus, the aim of the present study was to investigate structural changes in the penile corpus cavernosum of prepubertal chronically stressed rats.

Materials and Methods

Fifteen 4-week-old Wistar male rats created in our laboratory were included in the study. The animals were kept with their mothers until the third week of life. The rats were kept in a room with a controlled temperature (25°C ± 1°C) and artificial dark-light cycle (lights on from 7:00 AM to 7:00 PM) and were fed standard rat chow and water ad libitum. The rats were weighed weekly until the day of death. All experiments were performed according to the Brazilian law for scientific use of animals, and this project was formally approved by the local ethics committee.

The rats were randomly assigned into 2 groups, control (C; n = 7) and stressed (S; n = 8). Group C was maintained under

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Table. *Impact of stress stimuli on morphometric values and testosterone serum concentrations*[a]

	Control	Stressed	*P*
Adrenal mass index, g/kg	0.1245 ± 0.015	0.1432 ± 0.017	.026
Testosterone concentration, ng/mL	0.1925 ± 0.110	0.0820 ± 0.016	.028
Final weight, g	231.3 ± 16.6	200.5 ± 10.14	.0007
Smooth muscle surface density, %	14.07 ± 3.885	8.980 ± 3.704	.0222
Conjunctive tissue surface density, %	53.66 ± 9.864	64.47 ± 2.777	.0106

[a] Data expressed as means ± SD.

standard conditions until age 9 weeks. Group S was subjected to stress stimuli daily until age 9 weeks.

Stress stimuli were performed by immobilization in a rigid opaque plastic cylinder that restrained the movements of the rats (Retana-Márquez et al, 2003). The cylinders, with different diameters and lengths, were adjusted weekly depending on the animal's weight. The animals did not experience any pain and were contained for 2 hours daily in the morning from the 28th to the 63rd day of life.

Adrenal mass index (weight of adrenal/body weight) and serum testosterone concentration (measured by radioimmunoassay) were used to assess the physiologic efficacy of the stress stimulus (Bauer et al, 2001; Hardy et al, 2005). At the 64th day of life, under deep anesthesia, the blood was collected by heart puncture and the serum was separated by centrifugation and used for testosterone level determination.

The rats were sacrificed by anesthetic overdose, and penis and adrenal glands were dissected, cleared of adipose tissue, and fixed in 4% buffered formaldehyde. The skin-denuded middle part of the penile shaft was processed for paraffin embedding and cross-sections of 5-μm thickness were obtained.

Quantitative analysis of corpus cavernosum smooth muscle and conjunctive tissue was performed in Masson trichrome–stained slices captured under ×200 magnification by an Olympus BX51 microscope with a coupled DP70 digital camera. A 100-point grid was superimposed over the images using the software Image J (National Institutes of Health, Bethesda, Maryland), and the point-counting method (Pereira-Sampaio et al, 2007) was used to objectively determine smooth muscle and conjunctive tissue surface density, expressed as percentage. Five different fields were selected from 5 nonadjacent slices, for a total of 25 images, or 2500 points, counted for each animal.

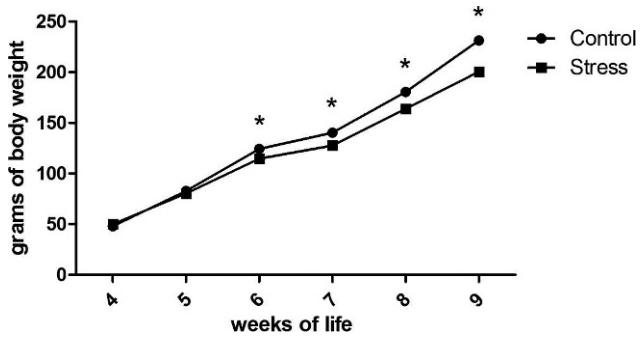

Figure 1. Graphic showing the body weight gain of prepubertal rats during restraint stress stimuli (from fourth to ninth week of life). Note that stressed animals had lower gain then control animals. Statistical difference (*) was noted from the sixth week of life until the end of the experiment.

Picrosirius red–stained slices were observed under polarized light to differentiate collagen types III (seen in green) and I (red/orange) (Montes, 1996).

The Student's *t* test was used for mean comparisons. In all cases, significance was set at a probability value of .05. All analyses were performed using GraphPad Prism software (La Jolla, California). All results are expressed as means ± SD.

Results

Increased adrenal mass index and decreased serum testosterone levels confirmed the efficacy of the stressor stimuli used for this research. For both parameters, significant differences were observed between the 2 groups (Table). In the group of stressed rats, the adrenal mass index was 15% higher and serum testosterone level was 57% lower than in the control group.

During our experiment, the stressed rats presented less body weight gain when compared with controls. This difference increased and became statistically significant at the second week of the experiment (sixth week of life) (Table; Figure 1). The mean body weight in stressed rats was 13.3% lower than that in controls.

Smooth muscle fiber surface density in corpora cavernosa of stressed animals decreased by 36% when compared with control animals. On the other hand, conjunctive tissue surface density was increased by 20% in stressed animals' erectile tissue. These numeric data are presented in the Table and illustrated in Figure 2.

When observed under polarized light in picosirius red–stained slices, the corpus cavernosum of stressed animals showed a higher predominance of red/orange collagen (indicating collagen type I), compared with control animals, which presented more green collagen (Figure 2).

Discussion

Penile erection requires neural transmission of proerectile impulses, an intact arterial blood supply, and functional erectile tissue in the corpus cavernosum. ED

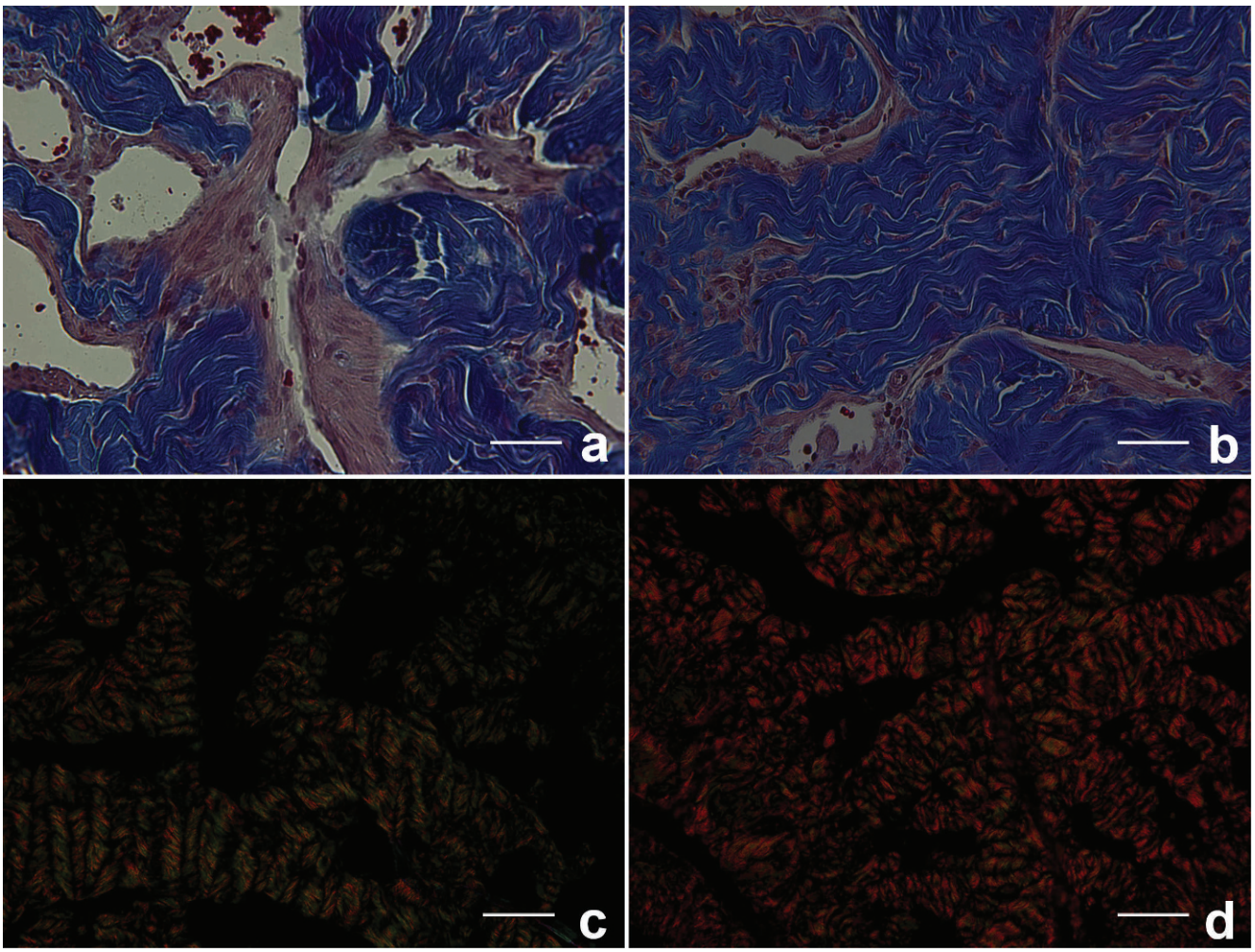

Figure 2. Photomicrographs of corpus cavernosum of control rats **(a** and **c**) and chronically stressed animals **(b** and **d**). Analyzing images **(a)** and **(b)**, we observed a reduction of smooth muscle fibers and an increase in conjunctive tissue in stressed animals **(c)** when compared with controls **(a)**. Masson trichrome, ×200. Analyzing images **(c)** and **(d)**, we observed a higher predominance of red/orange collagen in stressed animals **(d)** when compared with controls, which presented a predominance of green collagen. Picrosirius red under polarization, ×200. Scale bars = 100 μm. Color figure available online at www.andrologyjournal.org.

can develop from a defect in one of these tissues or from a defect in a combination of tissues. ED is classified as vasculogenic, neurogenic, hormonal, anatomic/structural, drug induced, or psychogenic (Lue, 2000; Hatzimouratidis et al, 2010).

From an anatomic aspect, the corpus cavernosum is the most important structure involved in erection and is mainly composed of smooth muscle fibers, conjunctive tissue, blood vessels, and vascular trabeculae. Conjunctive tissue must allow for elongation and increase in rigidity during erection and provide adequate resilience to return rapidly to the relaxed flaccid state after detumescence, whereas smooth muscle fibers should relax to maintain the erection by increasing the intracavernous pressure during erection, which could not be achieved by vascular mechanisms alone. Thus, to achieve normal penile function, an adequate percentage of smooth muscle fibers and connective tissue is required (Costa et al, 2006).

As seen in the stressed rats of the present study, a loss of smooth muscle fibers and an increase in conjunctive tissue (called penile fibrosis) in corpus cavernosum tissue are commonly present in patients with ED (El-Sakka and Yassin, 2010). Also, in stressed animals, the predominance of type I collagen fibers supports extracellular matrix remodeling and tissue fibrosis. Penile fibrosis is also related to aging, diabetes mellitus, cavernous nerve damage, and androgen deprivation (El-Sakka and Yassin, 2010). However, this is the first reported study showing evidence of stress as an etiology for penile fibrosis.

Although there are morphologic differences between human and rat corpus cavernosum that make this animal an imperfect model (Pinheiro et al, 2000), the

mechanisms of penile fibrosis seems to be similar because some diseases promote it in a similar manner (Kovanecz et al, 2009).

The effects of stress on sexual behavior and function have been previously demonstrated (Sato et al, 1996). Pubertal chronic stressed rats exhibited a 2-fold increased latency for the first mount when exposed to females in natural estrus (Almeida et al, 2000), and the relationship between ED and stress has also been demonstrated in humans (Bodie et al, 2003). An elegant study showed a high prevalence of work stress in men with ED alone or with associated hypogonadism. The authors found a strong association between work stress and lower testosterone levels (Guay et al, 2010).

Rats subjected to a similar model of restraint stress had impaired erectile response. This was investigated by physiologic measurements such as maximum intracavernous pressure and detumescence time after cavernous nerve electrical stimulation (Bal et al, 2009). However, the present study reports, to our knowledge, the first morphologic evidence of the effects of stress on penile structures.

Morphologic modifications seen in corpus cavernosum were most likely secondary to neuroendocrine implications of stress, especially the decrease in testosterone levels. It is well known that an adequate display of masculine sexual function depends mainly on testosterone, and testosterone's secretion is suppressed by stress (Retana-Márquez et al, 2003), as observed in our animals. Thus, stress may lead to ED with morphologic changes as a secondary effect.

Reduced testosterone concentration is mediated by the interference of chronic stress hypothalamus-pituitary-adrenal axis reducing the secretion of luteinizing hormone and gonadotropin-releasing hormone (Demura et al, 1989; López-Calderón et al, 1991). The increased levels of glucocorticoids also act in Leydig cell receptors, leading to a blockade of testosterone biosynthesis in the presence of normal luteinizing hormone levels (Orr and Mann, 1992).

Androgenic deficiency, with low levels of testosterone, is related to vascular disease and endothelial dysfunction (Traish et al, 2009). Also, testosterone activates nitric oxide synthase and acts as a vasodilator in the penis. Castrated rats have nitric oxide synthase activity reduced by 45%, and it has been demonstrated that testosterone replacement prevents such reduction (Chamness et al, 1995). Another role of testosterone in erection physiology is attenuating α-adrenergic vasoconstrictor activity in vascular smooth muscles of the corpus cavernosum and contributing to the penile venous occlusion mechanism that maintains erections (Mikhail, 2006).

It is possible that in the stressed rats, androgenic deficiency acts by different mechanisms, leading to ED and penile fibrosis. For limitations of this study, we must mention that the results should not be directly applied to humans because this study was experimental and performed in a model that is not ideal. Also, the stress stimuli was applied under controlled conditions that do not reflect the most common stressors of human lives.

We conclude that chronic stress in prepubertal rats led to reduced serum levels of testosterone and morphologic changes in corpus cavernosum, which may be related to ED.

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