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Effects of chronic restraint stress in the prostate of prepubertal and adult rats

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ABSTRACT

Purpose: To investigate the effects of chronic stress in the prostate of prepubertal and adult rats. **Methods:** Thirty-two male rats were assigned into four groups depending on the type of treatment (control or stressed) and the age at which stress was initiated (prepubertal or adult). Restraint stress stimuli were applied for six weeks. Stressed prepubertal and adult rats evaluated immediately after the last stress stimuli were named SP and SA groups, respectively. Age-matched rats were used as control groups (CP and CA). At the end of the experiment, the rats were euthanized, and prostate morphological parameters were evaluated and statistically compared. **Results:** Application of stress stimuli to the SP group resulted in reduced body weight, but no prostate morphological modification was noted. The SA group showed reduced testosterone level and prostatic epithelium surface density, in comparison to CA group. Further, the prostatic lumen surface density was increased in adult stressed animals, in comparison to adult controls. **Conclusion:** The stress stimuli promoted changes in hormonal and morphological parameters in the prostate of adult stressed rats. Prepubertal stressed animals did not presented modifications of prostate morphology.

Key words: Prostate. Rats.

Introduction

Stress is a biological condition to respond to an external stimulus. Although this condition is important enabling a better response to stressful situations, it involves several behavioral and physiological alterations[1]. Further when stress stimuli become persistent, a destructive effect on tissues are observed, and a set of morphological modifications are observed[1–3].

One very known physiological response associated with stress conditions is the over-activation of the hypothalamus-hypophysis-adrenal axis, resulting in an increased secretion of glucocorticoids. Also, the hypothalamus-hypophysis-gonadal axis is influenced, with reduced sexual hormones secretion in chronic stress situations[4].

Several studies have shown that the urogenital system organs have its morphology seriously altered by increased glucocorticoids levels and chronic stress[1,5–9]. However, few studies examinate the effects of stress on the prostate. Among then, some important experimental findings point to a relationship of stress stimuli and prostate cancer, with increased expression of cancer-related genes[10], and accelerated prostate cancer development[11,12].

The prostate morphology of stressed rats was superficially studied, with an apparent epithelium proliferation being observed in the ventral lobe[13]. But the prostatic stroma has not been studied, and the epithelium was not objectively assessed

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with morphometrical methods in stressed rodents. Further, it is already known that chronic stress can promote different alterations in the urogenital organs if induced during prepubertal or adult ages[5,6], but this has not been investigated in the prostate yet.

Thus, the objective of the present study was to evaluate the prostate alterations in prepubertal and adult Wistar rats submitted to chronic stress.

Methods

Animals

Thirty-two male Wistar rats were used in the experiments. The rats were kept in a temperature-controlled room (22 ± 1°C) with an artificial dark–light cycle (lights on from 7 a.m. to 7 p.m.), and fed standard rat chow and water ad libitum. The Animal Care and Use Committee of the Universidade Estadual do Rio de Janeiro approved the handling of the animals and the study design (protocol number CEUA/004/2015), which were used in accordance with national and international regulations. The authors complied with the ARRIVE guidelines.

Experimental design

Animals were assigned into four groups accordingly to age and treatment. SP (n = 7) was a group of 4-week-old prepubertal rats, and SA (n = 9) was a group of 10-week-old adult rats; both submitted to the stress stimuli. We compared each of these groups with age-matched control groups. CP (n = 8) was a control group of prepubertal rats, and CA (n = 8) was a control group of adult rats.

The SP and SA groups were submitted to chronic stress by the immobilization method[9]. Each animal was kept in a rigid opaque plastic tube for 2 hours daily, in the morning (from 9 to 11 a.m.), to restrain its movements during the six-week period. The plastic restraint tubes with different diameters and lengths were adjusted weekly depending on the rats' size. The behavior of the animals during, and immediately after the stress period were observed and recorded. Meanwhile, the control groups (CP and CA) were kept under normal conditions and not submitted to any stresses, but food and water were removed during the same period (2 hours in the morning). All animals were euthanized 24 hours after the last day of stress stimuli application, when the rats were 10 (SP and CP) and 16 (SA and CA) weeks old. Euthanasia was performed by isoflurane (Isofluorano, BioChimico, Itatiaia, RJ, Brazil) inhalation in an induction chamber.

Data collection

Just before death, body weight was measured, and blood was collected by heart puncture for the determination of testosterone levels using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cat. ADI-900-065, Enzo, New York, United States of America, sensitivity of 5.67 pg/mL)[14].

The ventral lobe of the prostate was dissected and fixed by immersion in formaldehyde 3.7% for at least 48 hours. Further, the samples were processed for paraffin embedding to obtain non-serial 5-µm-thick histological sections. Morphometric analyses were performed in hematoxylin and eosin (H&E) stained sections by a microscope (BX51, Olympus, Tokyo, Japan) coupled to a digital camera (DP70, Olympus). All images were saved in the tagged image file format (.tiff), at a resolution of 2,040 × 1,536 pixels[15].

The height of the acinar epithelium was measured in micrometers with the "straight line" tool of the ImageJ software (National Institutes of Health, Bethesda, Maryland, United States of America) in photomicrographs of 200× magnification. For this purpose, 125 measurements of the epithelium (most commonly, five measurements per histological field, from at least 25 histological fields) were performed for each rat[16,17].

The acinar area was measured in squared micrometers with the "polygon selection" tool of the ImageJ software in photomicrographs of 400× magnification. For this analysis, all acini that were completely observed in the histological field were measured[18]. At least, 25 histological fields per animal were studied.

The surface densities (Sv) of the lumen, epithelium and stroma were assessed by the point intercepts method[19,20]. Briefly, using the ImageJ software, a 100-point grid was superimposed over images under 400× magnification. Each structure touched by a point was counted, and its density was determined as a percentage of the analyzed field. For each rat, 25 fields were evaluated.

Statistical analysis

The means of the groups submitted to the stress stimuli were compared with its correspondent age-matched control group using the Student's t-test. All analyses were performed with the GraphPad Prism 5.0 software (GraphPad Software, San Diego, California, United States of America). Mean differences were considered significant when $p < 0.05$. All results were presented as mean ± standard deviation.

Results

All animals submitted to stress stimuli showed behavioral alterations during and immediately after the stress sessions. The behaviors include attempts to scape before entering the tube, tachypnea, and diarrhea inside the tube, and running after being removed from the tube to the boxes.

Effects of the stress stimuli in prepubertal rats

Body weight was reduced by 29 grams (14.1%; $p < 0.05$) in SP animals in comparison to their controls. No significant difference of serum testosterone levels was identified between SP and CP groups ($p = 0.23$). Also, no morphometrical differences were observed among these groups regarding epithelium height ($p = 0.55$), acinar area ($p = 0.27$), lumen Sv ($p = 0.89$), epithelium Sv ($p = 0.61$), and stroma Sv ($p = 0.49$). All numerical data of groups CP and SP are presented in Table 1.

Table 1 – Quantitative data of prepubertal rats submitted to chronic stress (SP) and its age matched control group (CP)*.

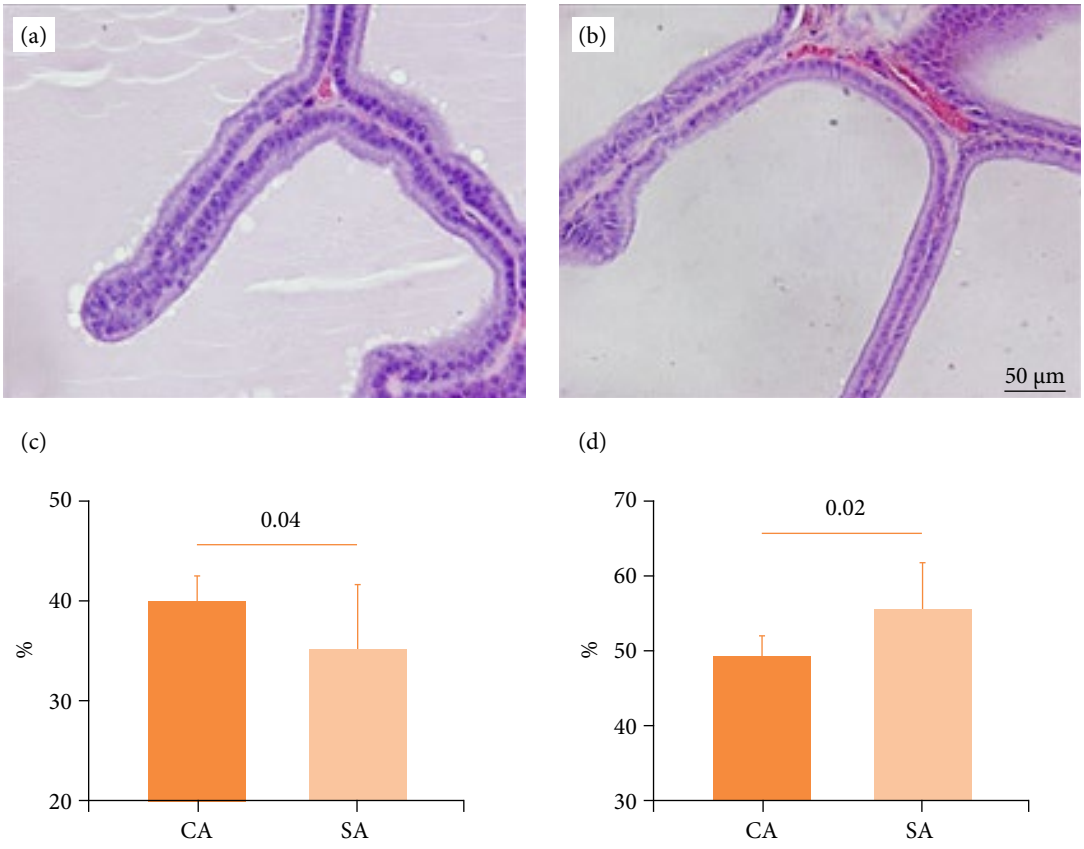
Parameters	CP	SP	*P*-value
Body weight (g)	206.23 ± 11.02	177.13 ± 15.85	< 0.01
Testosterone serum level (ng/mL)	13.00 ± 1.67	11.93 ± 1.21	0.23
Prostatic epithelium height (µm)	21.27 ± 2.39	20.57 ± 1.98	0.55
Prostatic acinar area ($\times 10^3$ µm^2)	50.88 ± 10.96	59.23 ± 11.32	0.27
Prostatic lumen surface density (%)	48.64 ± 9.24	48.04 ± 8.07	0.89
Prostatic epithelium surface density (%)	45.30 ± 5.85	43.51 ± 7.36	0.61
Prostatic stroma surface density (%)	6.84 ± 1.08	7.23 ± 1.07	0.49

*Data expressed as mean ± standard deviation. Source: Elaborated by the authors.

Effects of the stress stimuli in adult rats

No difference in body weight was observed when comparing CA with SA ($p = 0.86$). The testosterone serum levels of stressed animals showed reduction of 2.42 ng/mL, what represents 21.5% of this hormonal level ($p = 0.04$).

In respect to epithelium height ($p = 0.58$), acinar area ($p = 0.10$), and stroma Sv ($p = 0.61$), no statistical difference was observed among groups CA and SA. Meanwhile, stressed animals showed an increase of 12.0% in lumen Sv ($p = 0.02$), in comparison to its age-matched control group. Further, the prostatic epithelium Sv of group SA was reduced by 12.6% ($p = 0.04$) in comparison to CA (Fig. 1). All numerical data of groups CP and SP are presented in Table 2.



Source: Elaborated by the authors.

Figure 1 – Illustrative images from control and stressed rats. **(a)** Photomicrography of prostate from adult control rat (group CA); hematoxylin and eosin, 400×. **(b)** Photomicrography of prostate from adult stressed rat (group SA); hematoxylin and eosin, 400×. **(c)** Graphic showing the reduction of prostatic epithelium surface density in group SA in comparison to group CA. **(d)** Graphic showing the augmented prostatic lumen surface density in group SA in comparison to group CA.

Table 2 – Quantitative data of adult rats submitted to chronic stress (SA) and its age matched control group (CA)*.

	CA	SA	*p*-value
Body weight (g)	353.21 ± 32.80	342.37 ± 28.39	0.86
Testosterone serum level (ng/mL)	11.25 ± 2.14	8.83 ± 1.53	0.04
Prostatic epithelium height (µm)	19.20 ± 2.15	19.82 ± 2.25	0.58
Prostatic acinar area ($\times 10^3\ \mu m^2$)	62.36 ± 5.73	74.52 ± 19.14	0.10
Prostatic lumen surface density (%)	49.65 ± 2.12	55.62 ± 6.39	0.02
Prostatic epithelium surface density (%)	40.30 ± 2.14	35.22 ± 6.18	0.04
Prostatic stroma Sv (%)	8.67 ± 0.49	8.07 ± 1.74	0.35

*Data expressed as mean ± standard deviation. Source: Elaborated by the authors.

Discussion

The present study shows that adult rats submitted to chronic stress shows prostatic and hormonal alterations, with testosterone reduction, prostatic acinar area and lumen surface density augmentation and prostatic epithelium reduction. Meanwhile, no statistical difference (in testosterone levels and prostate morphology) was observed in prepubertal rats submitted to chronic stress.

Chronic stress is known to activate the hypothalamic-pituitary-adrenal axis, with consequences on the hypothalamic-pituitary-gonadal axis. One main result of this endocrine disturbance is the reduction of sexual hormones production/secretion in stressed individuals[21]. Our group have already shown that this model of chronic stress reduces the testosterone serum levels in chronically stressed rats[5,6,9], similarly of what was observed in the current experiment. This is in accordance with what Selye proposed when studying the mechanisms of stress. During stressful situations, the whole organism prepares to deal with the stressor, and physiological mechanisms not required in that immediately time are suppressed[22]. The reproductive system is one example of a physiological system that should theoretically be suppressed in a stressful situation[23]. Glucocorticoids can also interfere with testosterone levels directly, and it is known that the hypothalamic–pituitary–adrenal and the hypothalamic–pituitary–gonadal axis interact with each other[21].

The testosterone reduction led to penile and testicular morphological modifications[5,6,9], but the prostate gland was not studied yet. As an androgen-dependent organ, the prostate is very influenceable by sexual hormones, and it was expected that the prostate gland would be very modified by chronic stress. Surprisingly, it was observed only discrete morphological modifications in our stressed animals' prostate. More prolonged periods of stress would be interesting to verify if more pronounced modifications could be observed in adult, and (specially) in prepubertal animals. This study used the same protocol (2 hours per day, for six weeks) used before with interesting results in other urogenital organs[1,5–7,9]. Thus, it is possible to believe that the prostate may be a less stress-affected organ.

In adult rats submitted to chronic stress, a reduction of prostatic epithelium surface density was observed. This can be a direct effect of stress on the epithelial cells or (more probably) an indirect effect, i.e., a consequence of the reduced testosterone level. Paradoxically, the epithelium height was not modified in stressed rats. This could be explained by the reduced number of epithelial folds in stressed animals, leading to a reduced surface occupied by the epithelium, without epithelial height modification. Another finding in stressed animals was the raised luminal surface density, which was probably a consequence of the epithelium Sv reduction.

The study of Mukerjee and Rajan[24] showed very comparable findings with those from the present study. In their study, prepubertal rats stressed by maternal deprivation or foot-shock showed reduced epithelium volume in comparison to control animals. In the study of Morone Pinto et al.[25], chronic stress (induced pharmacologically) also negatively impacted the prostatic epithelium, reducing its height. Although these authors did not measure the Sv of epithelium, these findings corroborate the outcomes that chronic stress negatively impacts the prostate epithelium.

One interesting result of the current study is that adult animals showed more prominent morphological alterations in the prostate when compared to prepubertal rats. Actually, no statistically significant difference was observed in the prostate of prepubertally stressed rats, in comparison to controls. One possible reason would be the fact that prepubertal individuals are more adaptable to different situations than adults. Pubertal phase is mainly a phase of important changes and adaptation. Further, prepubertal animals may not suffer alterations of the hypothalamic-pituitary-gonadal axis as testicles are not fully developed. This could explain the non-statistically reduced testosterone levels in group SP. These findings are similar to our previous studies which compared the stress in prepubertal and adult rats[5,6]. When studying the testes and the penis, we observed that the damages promoted by chronic stress are more significant when stress stimuli is induced in adulthood than during prepubertal phase. The only alteration observed in prepubertal animals submitted to chronic stress was a body weight reduction. This could be explained by an accentuated energy expenditure caused by stress, although future studies should be performed to better elucidate this finding.

In the present study, rats were used to evaluate the effects of chronic stress on the prostate. A limitation of this study is the animal model used, as the results may not reflect the actual effects in men. Even considering that the prostatic ventral lobe of rats is a good model for morphological studies, several differences are observed among the prostate of human and rats.

Further studies on the effects of chronic stress are warranted. Different types, intensity, and time of the stressor stimulus, as well as the age of animals, would be necessary to be studied before a definitive decision regarding the impact of stress on the prostate. Although corticosterone analysis was not performed in this study, which may be considered a limitation of

the study, the stress induction method is a well stablished protocol. Our findings showed that chronic stress applied during adulthood causes prostatic morphological alterations. However, it is not known if these alterations could be reversed upon withdrawal of the stressor stimulus or prevented by some therapy. One possible limitation regarding all stress models is the adaptation to the stress stimuli. As behavior alterations is noted in all animals of stressed groups, throughout the experiment, it is possible to believe that this was not the case in the present study.

Conclusion

Based on the results obtained in the present study, it was possible to state that the stress stimuli promote hormonal disbalances and morphological alterations in the prostate of adult rats. Meanwhile, animals stressed during prepubertal phase did not presented modifications of prostate morphology or testosterone secretion alterations.

Conflict of interest

Nothing to declare.

Authors' contributions

Conception: Costa W, Sampaio F, Pereira-Sampaio M and de Souza D. **Acquisition of data:** Procópio I, Ribeiro C, Marchon R, Buys-Gonçalves G and de Souza D. **Manuscript writing:** Procópio I, Costa W, Pereira-Sampaio M and de Souza D. **Critical revision:** Ribeiro C, Marchon R, Buys-Gonçalves G and Sampaio F. **Final approval the version to be published:** Procópio I, Ribeiro C, Marchon R, Costa W, Buys-Gonçalves G, Sampaio F, Pereira-Sampaio M and de Souza D.

Data availability statement

All data sets were generated or analyzed in the current study.

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About the authors

Procópio IM is master.

Ribeiro CT, Marcho RG, Costa WS, Buys-Gonçalves GF, Sampaio FJB, Pereira-Sampaio MA and de Souza DB are PhDs.

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