The distal vas deferens of spontaneously hypertensive rats – a histomorphometric evaluation

Gabriela Faria Buys-Gonçalves¹, Marcello Henrique Araújo da Silva¹, Fernanda Martins Gonçalves¹, Gabrielly Alves da Rocha¹, Marco Aurélio Pereira-Sampaio¹,², Diogo Benchimol de Souza¹

ABSTRACT

Introduction: The spontaneously hypertensive rats strain are widely used as a model for investigation of hypertension involving ejaculatory function and its relevant structures. There are no reports of this rats' vas deferens histomorphometry or their comparison with Wistar Kyoto. Material and Methods: Fourteen male rats (4 months old) were divided into 2 groups: WKY - group composed of Wistar Kyoto rats and SHR - composed of spontaneously hypertensive rats. Mean arterial pressure was measured weekly. At age of 4 months, rats were euthanized. The right vas deferens were collected and processed. The area without adventitial tunica, the muscular tunica area, height of the pseudostratified epithelium with and without the stereocilia, as well as the height of the stereocilia were measured. The unpaired Student T test (parametric data) or the Mann-Whitney test (non-parametric data) were used to compare the results and they were considered significant when p < 0.05. Results: The mean arterial pressure was 59.4% higher in SHR. The area without the tunica adventitia was 15.5% lower in SHR, as well as muscular tunica area. The height of the pseudostratified epithelium with stereocilia was 26.4% lower in SHR, as well as the height of the pseudostratified epithelium without stereocilia in 23.1% and the height of the stereocilia in 36.2%. Conclusions: The distal vas deferens of spontaneously hypertensive rats present histomorphometric differences when compared to the control strain. Such data should be taken into account when using the model in studies that aim to evaluate the ejaculatory function.

Keywords: animal model, histomorphometry, hypertension, rat, vas deferens

INTRODUCTION

Recent studies have shown that hypertension is related to a decrease in ejaculatory function in men [1]. This also occurs in relation to erectile dysfunction [1, 2] and subsequently causes a decrease in the quality of the sexual activity [3]. Therefore, ejaculatory dysfunction should be considered in the initial evaluation and in the clinical management of hypertensive men [1].

Normal ejaculation consists of two distinct phases: emission and expulsion. The emission involves the secretion of seminal fluid from the accessory sex glands and contraction of the seminal tract from the epididymis to the prostate [4]. The correct functioning of such structures must occur in order for the spermatozoa to be satisfactorily transported up to the prostatic urethra [1], their correct function also depends on the tissue integrity of these organs. One of these is the vas deferens, a

¹Urogenital Research Unit, State University of Rio de Janeiro, Rio de Janeiro, Brazil.
²Department of Morphology, Fluminense Federal University, Niterói, Brazil.
Corresponding author: Diogo Benchimol de Souza, PhD - diogobenchimol@gmail.com
Rats’ vas deferens histomorphometry

Tubular organ that connects the tail of the epididymis to the ejaculatory duct, being responsible for the conduction of the male gametes until the same during the emission.

The use of animal models, especially the murine model, is common for experiments that investigate hypotheses related to sexual dysfunction [5, 6]. Spontaneously Hypertensive Rats (SHR) develops experimentally induced hypertension [7] and are widely used as a model for investigating the disease involving ejaculatory function and its relevant structures [8, 9].

The vas deferens of the mouse can be anatomically divided into two regions: proximal and distal. The proximal begins at the tail of the epididymis and ends at the point where the muscular lining of the duct begins to thicken. Because of its relatively thin layer, the proximal region is noticeably narrower than the distal one, which begins in the mid-length of the duct just before it enters the inguinal region [10].

Histologically, the proximal and distal regions can be differentiated based on the characteristics of the epithelium and muscular lining around the duct. Transverse sections of the distal region are circular due to the presence of thick muscle layers. In the junctional region between the proximal and distal vas deferens, the stereocylidised pseudostratified epithelium, which is evenly distributed over the submucosa, begins to show evagination that also encompasses the lamina propria and the blood vessels contained therein [10].

During the search of the main scientific platforms available, no reports were found on the histomorphometry of the vas deferens of SHR rats or their comparison with that of their Wistar Kyoto control, these being the objectives of the present study.

MATERIAL AND METHODS

All experiments were carried out in accordance with national and international laws on the scientific use of animals, and this project was formally approved by the Institutional Ethics Committee for animal experimentation.

Fourteen male rats (4 months old) were divided into two groups: WKY - group composed of seven Wistar Kyoto and SHR rats - composed of seven spontaneously hypertensive rats. The animals were fed standard rat chow, filtered water ad libitum and kept in a room with constant environmental conditions (room temperature 20 ± 2 °C, relative humidity 50 ± 10%, light-dark cycle of 12 hours). Mean arterial pressure was measured weekly from the time the mice completed 3 months (pubertal age) using a tail plethysmograph (V2.11, Insight, Ribeirão Preto, Brazil).

At 4 months of age, the animals were euthanized by anesthetic overdose (Isoflurane, BioChimico, Rio de Janeiro, Brazil). The right distal vas deferens were collected and fixed in 4% buffered formalin. Random samples from all fourteen distal vas deferens were processed for inclusion in paraffin, sectioned at 5 μm thick and stained with Masson's trichrome. The area without adventitial tunica and the muscular tunica area were estimated by specific delimitation in 10 photomicrographs per animal. The height of the pseudostratified epithelium with and without the stereocylls, as well as the height of the stereocilia were measured by linear tracing of the basal membrane to the apical cell (including or not the stereocylls) only in the inferior epithelial region between the characteristic duct evaginations distal deferens in twenty-five photomicrographs per animal.

The unpaired Student T test (parametric data) or the Mann-Whitney test (non-parametric data) were used to compare the results with the GraphPad Prism 5 program for Windows (GraphPad Software, San Diego, USA) and these were considered significant when the value of p <0.05.

RESULTS

All results are summarized in Table 1. The mean arterial pressure was 59.4% higher in the SRH group (Figure 1). The area without a tunica adventitia was 15.5% lower in the SHR group and the muscular tunica area was 15.5% lower in the SHR group (Figures 1 and 2). The height of the pseudostratified epithelium with stereocylls was 26.4% lower in the spontaneously hypertensive rats, as well as the height of the pseudostratified epithelium without stereocilia in 36.2% (Figure 3 and 4).
Table 1: Mean arterial pressure and mean stereological values of the distal region of the vas deferens in WKY and SHR groups.

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>153.4±24.4</td>
<td>258.4±21.3</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Area without the tunica adventitia (mm²)</td>
<td>2.2±0.2</td>
<td>1.9±0.2</td>
<td>0.02*</td>
</tr>
<tr>
<td>Area of the muscular layer (mm²)</td>
<td>2.0±0.2</td>
<td>1.7±0.2</td>
<td>0.04*</td>
</tr>
<tr>
<td>Height of the epithelium with stereocilia (µm)</td>
<td>62.0±7.4</td>
<td>45.6±13.7</td>
<td>0.04*</td>
</tr>
<tr>
<td>Height of the epithelium without stereocilia (µm)</td>
<td>46.2±4.3</td>
<td>35.5±9.1</td>
<td>0.01*</td>
</tr>
<tr>
<td>Height of the stereocilia (µm)</td>
<td>15.9±3.2</td>
<td>10.1±5.1</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± standard deviation. *: p value was significant (< 0.05).

Figure 1: Photomicrographs of transverse sections of the distal region of the right deferent duct of the two groups. WKY: Wistar Kyoto Group; SHR: Spontaneously Hypertensive Group. Masson's trichrome, 40x.

Figure 2: Graphic displaying the mean arterial pressure and stereological measurements of areas without the tunica adventitia and the area of the muscular layer. *means significant difference between groups (p < 0.05).
DISCUSSION

The systemic arterial pressure of the SHR group was significantly higher, which is also reported in previous studies using spontaneously hypertensive rats [7-9, 12-17].

The distal region of the duct in the animals used in our study presented histological conformation compatible with the description of Hamilton and Cooper (1978), with cross-sections of the distal circular region, with presence of thick smooth muscle layers and evaginations that also include the lamina propria and the blood vessels contained therein [10].

The area of the vas deferens without a tunica adventitia and muscular tunica area were smaller in the SHR group, the result of the first measurement being an apparent consequence of the second. The α1-adrenoceptors are responsible for the mechanisms of smooth muscle contraction, which are fundamental for the occurrence of the emission and are widely distributed in all the participating organs of the same, for example, the vas deferens, both in the rat [3, 8, 9] and in the man [1, 11].

There are reports of alterations in the properties of these receptors in SHR [12], and among them there are studies involving the vas deferens, bringing the information that, in SHR, the
expression of α1-adrenoceptors is increased in relation to the control group [13-16].

The SHR model has high sensitivity to sympathetic autonomic nervous system stimuli [17,18] and sympathomimetics [9,12], which increases the responsiveness of α1-adrenoceptors and smooth muscle contraction. Therefore, the decrease in muscle tunica and, consequently, in the area without the tunica adventitia found in our work seem to reflect this condition of the spontaneously hypertensive lineage.

The development and maintenance of the epithelium of the vas deferens and the epididymis is dependent on the constant stimulation of testosterone [19,20]. However, although the SHR model has high levels of testosterone in relation to its control [9], the concentration increase of this sex steroid seems to cause loss of its effectiveness in the epithelium of the male genital organs [21].

The development and maintenance of the epithelium of the vas deferens and the epididymis is dependent on the constant stimulation of testosterone [19,20]. However, although the SHR model has high levels of testosterone in relation to its control [9], the concentration increase of this sex steroid seems to cause loss of its effectiveness in the epithelium of the male genital organs [21]. Taking these data into consideration, we can relate them to the decrease of the height of the pseudostratified epithelium with and without the stereocyls, as well as the height of these cellular stretches in the SHR group of the present study.

The spontaneously hypertensive rat lineage is often used as an experimental model for ejaculatory dysfunction and hypertension [8,9,12,13], and the histomorphometric results of a structure such as the vas deferens (which plays an important role during the emission) contained in our study may contribute to the discussion and understanding of future research using the SHR model.

CONCLUSIONS

The distal vas deferens of spontaneously hypertensive rats presented histomorphometric differences when compared to the normotensive control strain. Such data should be taken into account when using this model in studies that aim to evaluate the vas deferens and ejaculatory function.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This study was supported by funds from the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), from the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) and from the “Fundação Carlos Chagas Filho para Pesquisa do Estado do Rio de Janeiro” (FAPERJ), Brazil.

REFERENCES


**RESUMO**

**O ducto deferente distal de ratos espontaneamente hipertensos – uma avaliação histomorfométrica**

**Introdução:** A linhagem de ratos espontaneamente hipertensiva é amplamente utilizada como modelo para investigação da hipertensão envolvendo a função ejaculatória e estruturas relevantes. Não há relatos da histomorfometria do ducto deferente deste modelo ou sua comparação com seu controle Wistar Kyoto.

**Material e Métodos:** Quatorze ratos machos (4 meses de idade) foram divididos em 2 grupos: WKY - grupo composto por ratos Wistar Kyoto e SHR - composto por ratos espontaneamente hipertensos. A pressão arterial média foi medida semanalmente. Aos 4 meses de idade, os ratos foram eutanasiados. Os ductos deferentes direitos foram coletados e processados. A área sem túnica adventícia, a área da túnica muscular, a altura do epitélio pseudoestratificado com e sem os estereocílios, bem como a altura dos estereocílios foram mensuradas. O teste T de Student não pareado (dados paramétricos) ou o teste de Mann-Whitney (dados não paramétricos) foram utilizados para comparar os resultados, que foram considerados significativos quando p<0,05.

**Resultados:** A pressão arterial média foi 59,4% maior no grupo SHR. A área sem a túnica albugínea foi 15,5% menor nos SHR, assim como a área da túnica muscular. A altura do epitélio pseudoestratificado com estereocílios foi 26,4% menor nos SHR, assim como a altura do epitélio pseudoestratificado sem estereocílios em 23,1% e a altura dos estereocílios em 36,2%.

**Conclusões:** Os
ductos deferentes distais de ratos espontaneamente hipertensos apresentam diferenças histomorfométricas quando comparados à linhagem controle. Tais dados devem ser levados em consideração se o modelo for utilizado em estudos que objetivem avaliar a função ejaculatória.

**Palavras-chave:** modelo animal, histomorfometria, hipertensão, rato, ducto deferente