Introduction

Testicles continually produce testosterone from puberty until the end of life. This production decreases after 40 years of age, and may reach levels below normal and cause the clinical condition known as androgen deficiency in aging males (ADAM). This clinical picture is manifested by mood fluctuations, reduced intellectual activity, a decrease in libido and quality of erections, depression, besides a decrease in muscle mass and strength. Populational aging creates challenges related to cardiovascular disturbances, decreased working capacity, and increased physical dependency. Androgen deficiency may accompany male aging and be a part of this picture. The literature is still controversial as to androgenic hormonal replacement in all aspects of the adrenal gland in animals with hypogonadism secondary to total bilateral epididymectomy and orchiectomy.
the rats in order to identify the anesthetic level and possible anesthetic complications.

After the operations, the animals were distributed into the following studies in order to answer the issues that were the objective of this project.

**Study A: morphology of the adrenal gland**

Thirty adult rats were distributed into three groups:
- Group 1 (n = 10), control;
- Group 2l (n = 10), orchietomy, rats euthanized on the 30th postoperative day, considered the immediate postoperative period (I);
- Group 2l (n = 10), orchietomy, rats euthanized after eight months postoperatively, during the period considered the late postoperative period (L).

Animals in Groups 1 and 2l were euthanized eight months after the operations in order to measure the serum levels of free testosterone by competitive assay and to evaluate macro- and micro-morphologies of the adrenal glands.

After the 30 day or eight-month follow-up period was concluded, the rats were anesthetized and weighed. In the morning, median laparotomies were performed by layers, and 5 ml sample of blood from the caudal vein was collected to determine the levels of free testosterone. Next, the adrenal glands were removed very carefully in order not to damage them. The animals were euthanized with deepened anesthesia.

The removed glands were processed for the histological study with hematoxylin-eosin to evaluate the general characteristics of the gland, and to establish the limit between the cortical and medullar layers. The thickness of the cortical layer was measured with the help of a lens with a 1 cm ruler marked in tenths of millimeters coupled to the left eyepiece of the optic microscope magnified 40 times.

During the microscopic examination, the adrenal was divided into four quadrants and in each one, the largest measurement of the cortical layer was considered. These four measurements of each gland were recorded for statistical analysis.

**Study B: assessment of blood glucose and lipid profile**

Forty rats, young (Y) and mature (M), were distributed into the following groups:
- Group 1 Y (n = 10), control, young rats;
- Group 1 A (n = 10), control, adult rats;
- Group 2 Y (n = 10), orchietomy, young rats;
- Group 2 A (n = 10), orchietomy, adult rats.

Blood was collected from all animals to measure blood glucose, total cholesterol and fractions, as well as triglycerides five months after the operations. The blood collection occurred after 12 hours of fasting with the animal anesthetized and supine. It was dissected right femoral vein and held vacuum system punch (Vacutainer®, Becton Dickinson, Brazil) 1 ml of blood in a sterile tube with separation gel and bottle covered with aluminum foil to protect from light.

**Automatic biochemical analyzer:** The samples were immediately processed. Values of total cholesterol (TC) and high-density lipoprotein (HDL) fraction were measured, as well as those of the triglycerides (TG), using the colorimetric dry chemistry method in an automated biochemistry analyzer VITROS® 950. Values of very low density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol were calculated following the Friedewald formula: LDL = CT – HDL – TG/5 and VLDL = TG/5. Blood glucose was measured by the colorimetric method (glucose-oxidase), with the same apparatus.

**Study C: mean arterial pressure**

Twenty-four adult rats were used, distributed into these groups:
- Group 1 (n = 12), control;
- Group 2 (n = 12), orchietomy.

Mean arterial pressure was measured in the animals before the operations and during the third post-operative month, in both groups, with a non-invasive method - plethysmograph located on the animal’s tail.

The general principle of the method is based on measurement of the amplitude of oscillation of the cuff pressure. Oscillations are caused by blood pressure pulses against the cuff. The average blood pressure is represented by oscillations with greater depth. For this to happen it is necessary to clearly tail vasodilation and thus heating of the animal. Pressure measurements were initiated with manual inflation of the cuff to 200 mmHg. Then, the minimum valve opening pressure gauge and slow reduction of the mercury column up to the middle tail blood pressure, manifested by free fall of the water column in parallel. The procedure lasted approximately 15 seconds.

The whole procedure took place in a calm environment after adaptation of the animal to the method. The training was necessary to reduce the variability of blood pressure measurements. It consisted of two daily sessions over three days simulating the definitive procedure (Whitesall 2004). Each session followed the same routine to heat the rat during 10 minutes in a wooden box to the approximate temperature of 33° C to 34° C. Then the animal was gently led to a tubular container with the following characteristics: 30 cm long, 10 cm or 15 cm in diameter as mouse size, and displaceable front ends to mouse containment and further fenestration for externalization the animal’s tail. The tail was adaptated to the plethysmograph with a mercury column, which defines the mean blood pressure and liquid level showing the cuff pressure oscillations. On the fourth day, they were made pressure measurements considering satisfactory oscillation of the liquid column that which manifested itself as free falling water. The dog’s movement artifacts and their desire not to position themselves appropriately for the measures were considered to invalidate results. The time for measuring the period followed 13 hours to 17 hours.

Measurements of adrenal thickness, body weight, free testosterone, blood glucose, and lipid profile were submitted to the
Kolmogorov-Smirnov test to determine the Gaussian distribution of the data, and to Bartlett’s test to identify variances. Next, variance analysis (ANOVA) was performed. In the results with a significant difference, post-testing was done with Tukey multiple comparisons in order to define which groups were different. Wilcoxon test analyzed the measurements of mean pre and post-operative arterial pressure values in Group 2. The results of post-operative arterial pressure of Groups 1 and 2 were compared with Mann-Whitney test. The significance level considered corresponded to the value of p < 0.05.

Results

All animals were euthanized at the end of the study and the data obtained were grouped as per the stages of the research project.

Study A: morphology of the adrenal gland

One non-orchiectomized adult animal (Group 1) was excluded from the analysis due to a failure in processing the blood sample for testosterone level determination.

In Group 2i, weight gain was smaller than in the Control Group (Table 1). Rats from Group 2i, sacrificed one month after orchiectomy, displayed the smallest weights among the three groups, but it should be considered that they died at a younger age, and therefore cannot be compared to the other two groups.

Table 1 shows lower serum levels of testosterone in orchiectomized rats relative to the Control. There was no difference between Groups 2i and 2l (p = 0.41).

Orchiectomy did not modify the histological pattern of the medullar and cortical adrenal layers, according to the histological preparation utilized, hematoxylin-eosin. As to the thickness of the adrenal cortex, ANOVA identified no difference between the groups (p = 0.54) as seen in Table 1.

Study B: assessment of blood glucose and lipid profile

Non-orchiectomized adult animals were excluded from the analysis (Group 1M) due to a failure in processing the blood samples, leading to hemolysis.

Orchiectomy did not modify serum levels of glucose, total cholesterol, and LDL and HDL fractions (p > 0.05 for all comparisons). However, when performed in young rats, orchiectomy reduced the blood levels of triglycerides and VLDL cholesterol (Table 2).

Study C: mean arterial pressure

Two animals from Group 2 were excluded due to technical difficulties in blood pressure measurement. These rats did not adapt to the method and were restless in the cage.

The orchiectomized animals displayed higher arterial pressures than those in the Control Group. There was a post-operative arterial pressure increase relative to the preoperative period in the rats from Group 2 (Table 3).

Discussion

Approximately 98% of the circulating testosterone is bound to plasma proteins, 40% is bound to a hepatic glycoprotein called sex

Table 1: Values (mean ± mean standard deviation) of body weight, free testosterone and adrenal gland thickness of rats in Groups 1, 2i and 2l.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g)</th>
<th>Free testosterone (pg/ml)</th>
<th>Adrenal thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 9)</td>
<td>546 ± 37</td>
<td>2,132 ± 948 **</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>Group 2i (n = 10)</td>
<td>315 ± 15</td>
<td>136 ± 39</td>
<td>0.70 ± 0.08</td>
</tr>
<tr>
<td>Group 2l (n = 10)</td>
<td>452 ± 21*</td>
<td>87 ± 14</td>
<td>0.71 ± 0.06</td>
</tr>
</tbody>
</table>

Group 1 (control): rats dead after 8 months
Group 2 (initial): orchiectomy, rats dead after 30 days
Group 2 (late): orchiectomy, rats dead after 8 months;
* different from mean of Group 1 (control) (p < 0.001)
** different from mean of other groups (p < 0.0001)

Table 2: Values (mean ± mean standard deviation, mg/dl) of blood biochemistry tests of rats collected five months after operations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>HDL</td>
<td>LDL</td>
</tr>
<tr>
<td>1 Y</td>
<td>125 ± 33</td>
<td>81 ± 12</td>
<td>25 ± 4.5</td>
</tr>
<tr>
<td>1 A</td>
<td>149 ± 25</td>
<td>77 ± 10</td>
<td>22 ± 4.9</td>
</tr>
<tr>
<td>2 Y</td>
<td>141 ± 27</td>
<td>86 ± 15</td>
<td>26 ± 4.7</td>
</tr>
<tr>
<td>2 A</td>
<td>130 ± 15</td>
<td>79.9 ± 11</td>
<td>23 ± 3.7</td>
</tr>
</tbody>
</table>

Group 1Y (young): n = 10, control, young rats.
Group 1A (adult): n = 8, control, adult rats.
Group 2Y (young): n = 10, orchiectomy, young rats.
Group 2A (adult): n = 10, orchiectomy, adult rats.
* different from mean as compared to Group 1Y (p = 0.005).
** different from mean as compared to Group 1Y (p = 0.005).
HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein
Values (mean ± mean standard deviation) of mean blood pressure (mmHg) in the tail of rats in groups 1 and 2.

<table>
<thead>
<tr>
<th>Preoperative</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>All rats</td>
<td>104.0 ± 2.9</td>
</tr>
<tr>
<td>Group 1 (n = 12)</td>
<td>107.8 ± 3.4*</td>
</tr>
<tr>
<td>Group 2 (n = 10)</td>
<td>121.1 ± 6.4**</td>
</tr>
</tbody>
</table>

Group 1: control and Group 2: orchietomy.
* Different from Group 2 (p = 0.034).
** Different from the preoperative level (p = 0.016).

In the study of body weight and adrenal gland morphology, eight months after the operations, the Control Group animals had gained more weight than the orchietomized rats. In the study performed by Vanderschueren et al., castration did not modify body weight more than the orchiectomized rats. In the study performed 9 months after the operations, the Control Group animals had gained weight similarly to the orchiectomized rats. In the study performed by Cherry et al., in animals with hypogonadism, and Parker et al., in individuals who died from trauma, found no difference in the total adrenal cortical thickness after hypogonadism, although they detected a proportional reduction of the reticular zone relative to the other cortical zones [16,17]. Since testosterone is produced in the testicles and adrenal glands, it is necessary to clarify if the adrenal gland takes on an endocrine compensatory role after orchietomy.

In addition, in this study, orchietomy in young rats reduced the blood levels of triglycerides and the VLDL cholesterol fraction. Such changes might be indicators of the influence of testosterone on the metabolism of lipids and cholesterol, which still need to be investigated [12,14]. There is an available evidence to support the role of hypertriglyceridemia in atherogenesis, and more recent report highlighting the role of hypertriglyceridemia in the VLDL fraction of cholesterol as well [15].

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flow. The pain caused by the arterial puncture may affect the heart rate and arterial pressure. In order to avoid these inconveniences, it was opted for a non-invasive method. Additionally, the accuracy of non-invasive blood pressure measurements does not differ from that of invasive methods in rats with normal pressure levels, as long as the examiner is qualified and the animal is trained, even considering that invasive methods may be better. A calm environment, adequate acclimatization, and gentle handling of the animals diminish interventional factors and increase reliability of measurements [18].

A literature review indicates that the administration of testosterone exacerbates the arterial pressure of previously hypertensive rats and that the castration of these animals reduces the arterial pressure [19,20]. Nevertheless, the same effect was not documented in rats with normal arterial pressure levels. In this study, castration was accompanied by an increase in blood pressure, a result similar to that found by Calhoun et al. [21].

Within Leydig cells, cholesterol is the precursor of sexual hormones and the main substrate for the synthesis of testosterone. During adolescence, the levels of cholesterol fractions HDL and LDL decrease. The levels of HDL are inversely related to testosterone in pubescent boys. These changes during sexual maturation indicate that the sexual hormones participate in the metabolism of the lipoproteins [22].

Some authors reported an increase in the LDL fraction in situations of testosterone deficiency in different species, including hypogonadal humans [23]. The decrease in HDL was observed in studies carried out in hypogonadal men and in castrated mice [24].

On the other hand, an increase or absence of variation of HDL levels also occurred in men with testosterone deficiency, and in castrated monkeys and mice [23].

Some animal studies showed that testosterone possesses a coronary vasodilation action on the thoracic aorta, pulmonary vasculature, and isolated mesenteric veins [25-28]. Testosterone not only induces vasodilation by the release of nitrous oxide, but also clearly acts in improving sensitivity of smooth vascular muscles to the action of this molecule [29].

Conclusions

Orchietomy reduces blood levels of triglycerides and VLDL cholesterol in young rats whereas in adult animals, arterial pressure increasing and smaller weight gain are verified.

Acknowledgements

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References


