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Effects of gonadectomy and steroid administration on histomorphometric properties of heart in male rabbit

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Abstract

The aim of the present study was to investigate effects of testosterone propionate (TP) on the histological properties of heart in gonadectomized rabbits. Fifteen New Zealand white rabbits, 2.5-month-old, were selected in this study. The animals were divided into 3 operation groups: sham-operated control animals (Co), gonadectomy animals (Go) and testosterone administration (TA). Gonadectomized rabbit hearts were compared with hearts of sham-operated control animals and their testosterone induction. Heart diameter and length of left and right epicardium in gonadectomized animals do not have any statistically significant difference than sham-operated rabbits. Heart weight, right and left ventricle thickness, and right and left cardiac myocardium decreased statically in gonadectomized animals as compared to the control group and increased significantly ($p < 0.05$) after testosterone administration.

Keywords: Gonadectomy; Rabbit; Steroid.

Introduction

The heart is a "complex three-dimensional fiber-wound structure with mechanical properties that are nonlinear, anisotropic, time varying and spatially inhomogeneous". Studies into the structure of the heart are at the cellular level, or histological studies that usually explore the myocyte structure, alignment and ionic pathways or overall fiber architecture (Greenbaum et al., 1981). Myocytes are target for the action of endogenous anabolic androgenic steroids (Smeets and Legros, 2004) and synthetic derivatives of the hormone testosterone (Evans, 2004). Androgen receptors have been well demonstrated in myocardium cells of hearts in baboon (McGill and Sheridan, 1981) and rat (Kneg et al., 1978) and confirmatory evidence conducted by Marsh et al. (1998) who showed the presence of androgen receptor transcripts in isolated cardiac myocytes from animals and humans. Sader et al. (2001) showed that steroids affect the function, size, shape and activity of the heart. Also, data indicate myocardium cells containing androgen receptors are distributed throughout the heart and major elastic and muscular arteries of the baboon (McGill et al., 1980). Steroid hormones may, in addition to their classical nuclear binding, exert some influence on cardiomyocytes via non-genomic, membrane mediated effects (Wilkinson et al., 1995); modulate cardiomyocyte growth and myosin-ATPase (Lengsfeld et al., 1988) by a range of effects mediated via androgens on

cardiomyocyte membrane that have been recently reported and including the stimulation of Ca fluxes and Ca dependent membrane transports (Christ et al., 1999).

Hormone-specific variation in cardiac function may be due to regulatory mechanisms induced by biological factors, since gonadectomy of rats has a profound effect on cardiac contractile performance. In heart perfusion studies, gonadectomized male and female rats display reduced cardiac function that can be reversed with hormone replacement (Scheuer et al., 1987). Gross finding related to the effects of gonadectomy on morphology of heart in rat shows that the males had lower body and heart weights than male sham operated rats (Schaible et al., 1984) and testosterone treated male rats showed a significant increase of the heart weight and of heart weight/body weight ratio (D'Antona et al., 2001). However, this study used surgical castration to induce gonadectomy in male rabbit. To our knowledge, no studies have examined the change of structure of the male heart during gonadectomy. The second purpose of this investigation was to investigate the effects of testosterone administration on cardiac morphology after gonadectomy in adult male rabbit.

Materials and Methods

Approval for all protocols of study was obtained from our Institutional Animal Care Committee and was in agreement with guidelines of Animal

Welfare Act. Fifteen adult male New Zealand white rabbits (3.2±0.4 kg BW; age, 2.5 months) were used in this research. Briefly, they were kept under similar conditions and housed in collective cages in a temperature-controlled room (23°C), received drinking water, and normal rodent pellets ad libitum throughout the entire period. The animals were randomly divided into three experimental groups: 1- sham-operated control (Co) (n=5); 2- gonadectomy (Go) (n=5); 3- testosterone administration (TA) (n=5). Those of control group were sham-operated and other groups were bilaterally orchidectomized under ketamin anesthesia (80 mg/kg body weight). After 4 weeks of orchidectomy, TA group was treated with Testosterone propionate (5mg/kg i.m.) for two weeks at seven day intervals without any detrimental effects. At the conclusion of the study after two months, the rabbits were profoundly anesthetized with sodium pentobarbital (50 mg/kg i.v.), euthanized with i.v. injection of KCl and were dissected. Abdominal viscera were eliminated and their hearts were carefully excised for morphological and histological studies. After trimming of excess tissue, the hearts were weighed and morphological properties such as length, diameter and thickness of the right and left ventricular walls in situ were determined. Heart length (mm) was measured from the anterior

aspect of the atria to the apex of the ventricles. Diameter (mm) of each heart was determined by making a whole cross section of coronary groove, cut surface down and the greatest diameter of the resulting outline of the heart were accepted as heart diameter. Thickness of ventricles was determined using a handheld micrometer, which was exact to 0.01 mm and number of myocytes were measured by point-counting planimetry at a magnification of 10 as previously described by Anversa et al. (1980). The tissue samples were immediately stabilized in 10% buffered formalin solution and embedded in paraffin. Then, tissue sections were stained with haematoxylin–eosin for study of the thickness of tissue layers of heart. All morphological measurements were analyzed among three groups with SPSS software and data were expressed as mean±SD and 95% confidence interval for intragroup comparisons. Differences with P<0.05 were regarded statistically significant.

Results

Table 1 shows the values of initial gross and histometric findings evaluated at the time of sacrifice. The results with reference to the analysis performed about principal effects of gonadectomy and those treated with androgen administration in comparison with control group on the rabbit hearts are presented in Table 2.

Table 1. Gross and histometric parameters of control group.

Parameter	Sex	Co	
		Mean±SD	Confidence Interval
Heart diameter (cm)		2.3±0.1	2.45-2.84
Heart length (cm)		3.21±0.17	3.12-3.41
Heart weight (g)		33±1.4	24.09-29.8
Right ventricle thickness (mm)		2.18±0.44	2.24-2.92
Left ventricle thickness (mm)		4.69±0.17	4.87-5.1
Right epicardium (µm)		45.17±1.86	48.27-63.47
Left epicardium (µm)		98.65±9.64	84.63-102.7
Right myocardium (µm)		1087.5±43.6	1077-1180.9
Left myocardium (µm)		3552.3±112.6	3348.5-3876.1

Table 2. Comparison of histometric structures of heart between gonadectomy and testosterone administration groups.

Sex Parameter	Go		TA	
	Mean±SD	Confidence Interval	Mean±SD	Confidence Interval
Heart diameter (cm)	2.1±0.14	1.4-1.7	2.2±0.13	2.33-2.75
Heart length (cm)	3.18±0.21	2.76-3.12	3.11±0.17	3.06-3.1
Heart weight (g)	29±1.04	21.53-25.46	32±1.1	23.19-28.8
Right ventricle thickness (mm)	1.87±0.06	1.93-2.1	2.17±0.12	2.02-2.63
Left ventricle thickness (mm)	3.87±0.1	3.1-4.6	4.1±0.1	4.14-4.78
Right epicardium (µm)	44.6±2.31	46.3-62.7	44.87±1.46	47.43-62.87
Left epicardium (µm)	97.14±8.73	80.76-98.43	97.86±8.3	82.76-100.43
Right myocardium (µm)	979.4±34.64	1065-1120.4	1055.1±31.1	1045-1140.5
Left myocardium (µm)	3112.2±102.7	3242.1-3787.6	3324.4±89.3	3278.5-3685.4

Stereological measurements showed that there was not any statistically significant difference in heart diameter and length plus left and right epicardium between Go and TA groups with respect to Co. Heart weight decreased statically in Go animals in comparison to the Co ($p<0.05$) and increased after testosterone administration and this difference was significant ($p<0.05$). In the Go group, right and left ventricle thickness, and right and left myocardium decreased statistically ($p<0.05$). Results obtained in TA group showed that androgen treatment of Go group reversed all the morphometric changes seen in Go group. The right ventricle thickness, left ventricle thickness, right myocardium and left myocardium were higher than in the Go group ($p<0.05$).

Discussion

Myocardial cells are targets for the action of testosterone and testosterone-like steroids (D'Antona et al., 2001). Under different situations, circulating testosterone levels altered frequently in human males. Thus, a reduction in circulating androgens may alter functional expression of cardiac myocytes (Golden et al., 2002). As expected, gonadectomy was associated with a marked decrease in

testosterone serum level, which was brought back to control values by the hormonal replacement. On the other hand, testosterone supplementation was attached with a striking increase in testosterone serum concentration (D'Antona et al., 2001). Gonadectomy is a well-established method to evaluate hormone effects (androgens) in animals (Scheuer et al., 1990).

The present study showed that heart weight, right and left ventricle thickness as well as right and left myocardium were decreased respectively (by 4%, 0.31%, 0.82%, 108.2% and 440.1% , $p<0.05$) compared to Co rabbits. Testosterone administration of Co animals reversed these changes to the pre-gonadectomy state. These observed morphologic changes show that the gonadectomy induced decrease in mass of cardiac myocytes and the testosterone administration reversed it. The observed changes of heart structure after gonadectomy and androgen administration could be due to the direct action of male gonadal androgens on heart muscle cells. Gonadectomy decreases hypertrophy of heart myocytes by decreasing mRNA levels for the androgen receptor, $\text{Na}^+/\text{Ca}^{2+}$ exchanger, L-type calcium channel, and 1-adrenergic receptor (Golden et al., 2002) and increased cardiac mass is the result of the

development of larger myocytes (Anversa et al., 1980) that were observed during testosterone treatment. Androgen-receptor has been detected in male adult rat cardiac myocytes, neonatal rat myocytes, dog heart, and juvenile human heart (Marsh et al., 1998). According to these findings, it can be stated that the heart weight, right and left ventricular thickness, and right and left myocardial thickness increased after androgen administration.

D'Antona et al. (2001) demonstrated that testosterone treated rats showed a significant increase of the heart weight and of heart weight/body weight ratio and a major finding of this investigation was that both castrated and testosterone overloaded rats exhibited an increased susceptibility to fibrillation. Therefore, it was concluded that gonadectomy has a partially reversible inhibitory effect on the heart in male rabbit.

Conclusion

Heart weight, right and left ventricle thickness, and right and left cardiac myocardium decreased statically in gonadectomized animals in comparison to the control group and increased after testosterone administration.

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