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Effect of Orchidectomy and Chronic Androgen Administration on Thyroid Gland in Adult Male Dogs: a Light and Electron Microscopic Study

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Abstract: This study was to evaluate the effect of orchidectomy and orchidectomy followed by chronic treatment with testosterone propionate on thyroid gland structure in male dogs. Twelve adult male dogs were divided into the sham operated control group (SO), orchidectomized group (Orx) and orchidectomized group treated with testosterone propionate (Orx TP). Thyroid tissue was investigated at both light and electron microscopic levels. In all three groups, both the peripheral and central regions of the thyroid followed similar patterns of histochemical, ultrastructural and stereological changes. Compared to SO dogs, the relative volume densities of the follicles, colloid and epithelium were decreased and that of the interstitium was increased in Orx dogs. The height of follicular cells also decreased significantly in Orx dogs in comparison to SO dogs. Chronic TP treatement of Orx dogs reversed these changes to the pre-orchidectomy state. At ultrastructural level, the thyroid follicular cells of Orx dogs were characterized by decreased number of mitochondria, free ribosomes, lysosomes, colloid droplets and secretory vesicles, apical microvilli and lateral and basal cell interdigitations. Chronic TP treatment of Orx dogs reversed all the ultrastructural changes seen in follicular cells of Orx dogs except those related to the cell membrane.

Key words: Gonadectomy · Thyroid follicular cells · Histomorphometry · Steroid hormones

INTRODUCTION

Andropause is commonly referred to as partial androgen deficiency and defined as an age-related decline in serum testosterone levels in older men. Symptoms associated with low testosterone levels in the aging male include low libido (with or without erectile dysfunction) [1]; decreased strength, energy, or stamina [2]; increased irritability or decreased enjoyment of life [3]; and alterations in certain components of cognitive function [4].

Androgen has a direct influence on thyroid cell proliferation [5-7]. Presence of androgen receptors has been reported in thyroid tissue of rat, primate and human [5, 8, 9]. Analysis of 3206 dogs diagnosed with hypothyroidism revealed an increased risk both in gonadectomised female and male dogs [10]. Accordingly, on the basis of clinical data, gonadectomy has been reported to be the most significant gender-associated risk factor for the development of hypothyroidism in dogs [11]. Castration was found to significantly decrease the incidence of thyroid tumors, as well as the mean concentration of TSH in rats treated with irradiation or carcinogen [12, 13], whereas testosterone replacement therapy was associated with both increased tumor yield and serum TSH [14]. However, little is known about the effects of orchidectomy and androgen replacement on histological organization of thyroid gland. The aim of this study was to examine the effects of gonadectomy and subsequent chronic testosterone administration on morphological and functional feautures of the thyroid gland in the adult male dogs.

MATERIALS AND METHODS

Twelve male Persian sheep dogs aged 1.5 years, an age when the thyroid metabolism is in an adult status, were housed in stainless steel cages. They were given water ad libitum and 250 g of laboratory dog food daily.

The animals were divided into three groups (n=4). Two groups were bilaterally orchidectomized (Orx) under anesthesia with a combination of ketamine hydrochloride 25 mg/kg and xylazine 10 mg/kg (Bayer Co., Ltd). Animals of the control group underwent sham operations (SO).

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One month after orchidectomy, one Orx group was treated i.m with 5-milligram testosterone propionate, obtained from ICN Galenika Pharmaceuticals, Belgrade, Serbia. The testosterone propionate was dissolved in sterile olive oil. It was administrated for eight weeks, at five day intervals. The other Orx group and the SO group were given a vehicle i.m injection according to the same schedule.

The animals were treated humanly during the test period and were euthanized 24 h after the last treatment whit an overdose of anesthetic formed by a mixture of ketamine hydrocloryde 20 mg/kg and xylazyne hydrochloride (1.5 mg/kg). The thyroids were excised followed by precise cervical dissection. Tissue segments from the same regions of both peripheral and central regions of right thyroid lobe were removed and immediately fixed by immersion in 10% neutral buffered formalin for light microscopy or 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for electron microscopy.

Paraffin-embedded sections were cut at 5 μm and stained according to haematoxylin and eosin (H&E). Small pieces (cubes of approximately 1 mm) of the previously removed segments of the glands were fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for two hours. The pieces were washed with buffer, postfixed in 1% osmium tetroxide in buffer, dehydrated with ethanol and embedded in resin. Ultrathin (70-90 nm thick) sections of the thyroid glands were cut and mounted on 200 mesh copper grids and stained with uranyle acetate and lead citrate. Stereological measurements were performed using a point-counting method and relative volumes (Vv) of follicles, epithelium, colloid and interstitium were measured using method described by Šošić-Jurjević *et al.* [15].

All stereological results were statistically evaluated by One- way ANOVA. Results are reported as mean ± SEM with a significance level of 0.05.

RESULTS

In the SO group, both the peripheral and central regions of the thyroid were composed of a mixture of large and small follicles. The follicles were spherical structures, each consisting of a central core of colloid surrounded by a single layer of epithelial cells and enclosed in a basal lamina (Figure 1a). Each follicular cell in both large and small follicles, showed a basally located nucleous, prominent rough endoplasmic reticulum, a supranuclear Golgi complex and numerous mitochondria, free ribosomes, lysosomes and colloid droplets. There were numerous secretory vesicles above the Golgi complex and short apical microvilli projecting into the colloid. Lysosomes were evenly distributed in follicular cells. The lateral cell surfaces exhibit elaborate interdigitations and the basal cell surfaces possess extensive infoldings (Figure 2a).

Results obtained from stereological measurements are shown in Table 1. No statistically significant differences were detected in the stereological parameters between the peripheral and central thyroid regions.

In the SO group, the volume densities of the follicles, epithelium, interstitium and colloid of the whole thyroid gland were 88±4, 17.5±3, 12±3 and 70.5±5 respectively, whereas the epithelial height was 9.2±1.1.

In the Orx group, both large and small thyroid follicles were considerably smaller than those observed in sham operated control group. Both peripheral and central follicles showed signs of decreased activity.

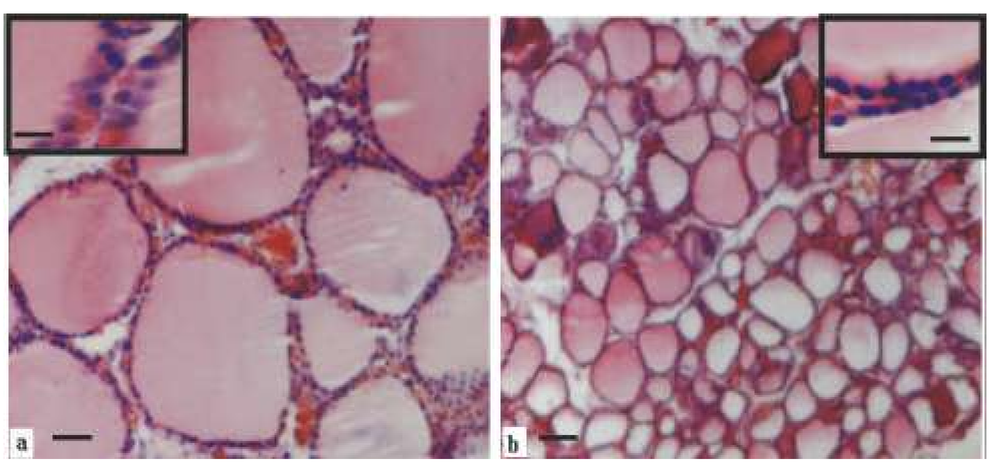

Fig. 1: a, The thyroid gland of a control SO adult male dog (H&E; bar=60 μm). The inset micrograph shows the thyroid follicular wall at higher magnification (bar= 14 μm). b, The thyroid follicles of an Orx male dog (H&E; bar=110 μm). The follicular wall at higher magnification (bar=18 μ

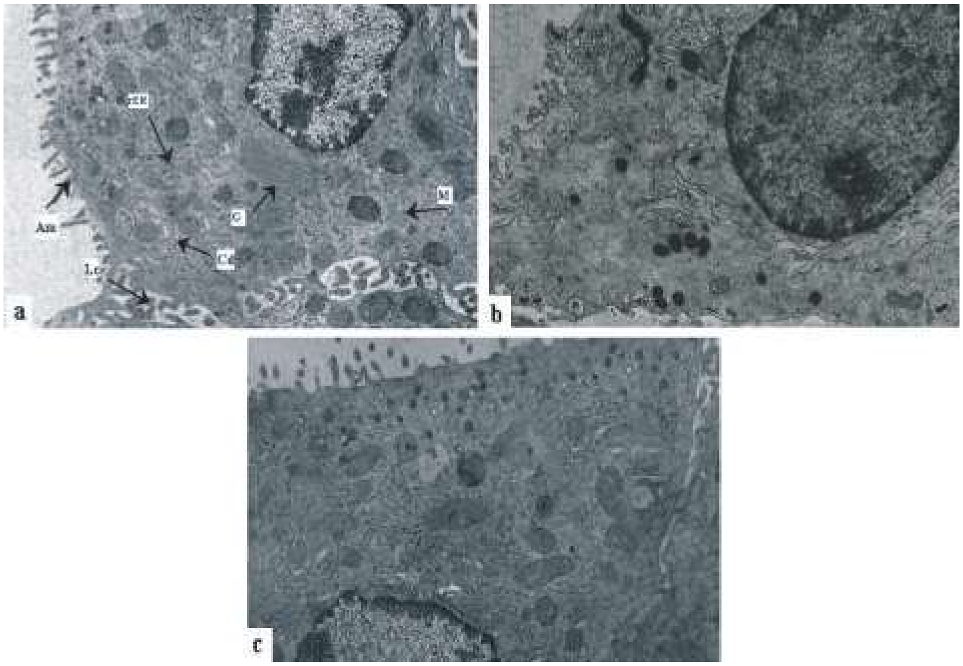

Fig. 2: a. Electron micrograph showing a follicular cell of the thyroid gland of a control SO adult male dog. Note the interdigitations of the lateral cell margin (Lc) and apical microvilli (Am). Rough endoplasmic reticulum (rER), a supranuclear Golgi complex (G). and numerous mitochondria (M), free ribosomes, lysosomes and colloid droplets (Cd) are among the usual organelles (× 13400). b, A follicular cell ofthyroid gland of a Orx dog. Note the decrease number of cytoplasmic organelles, apical microvilli and lateral cell interdigitations (× 13400). c, A follicular cell of thyroid gland of a Orx TP dog. TP treatment restores all the ultrastructural changes seen in follicular cells of Orx dogs except those related to the cell membrane (× 13400)

Table 1: Morphometric parameters for the peripheral and central thyroid zones in control (SO), orchidectomized (Orx) and orchidectomized TP-treared (Orx TP) adult male dogs

		Parameters				
Groups		Follicle *Vv (%)	Epithelium Vv (%)	Interstitium Vv (%)	Colloid Vv (%)	Height of follicular cells (μm)
SO	Peripheral	87±5	18±3	13±2	69±5	9.4±0.8
	Central	89±4	17±2	11±3	72±4	9±1.1
Orx	Peripheral	54±3	11±2	46±6	43±3	6.7±0.4
	Central	51±4	10±2	49±5	41±4	6.5±0.3
Orx - TP	Peripheral	86±4	16±3	14±2	70±4	9±0.9
	Central	91±4	18±4	9±4	73±3	9.2±0.7

The values are means ± SD (N=4); *Vv: Volume density; μm: micrometer

In all follicular cells, the nuclei were located in about the center of cells. The colloid appears denser and more eosinophilic (Figure 1b). The microvilli of the apical membrane were markedly reduced. The lateral cell margins showed less interdigitations and the basal membrane had fewer infoldings. The Golgi complex and rough endoplasmic reticulum were reduced and the mitochondria and free ribosomes were less in number (Figure 2b).

Stereological measurements showed that in both peripheral and central thyroid regions, the volume densities of the follicles, colloid and epithelium decreased ($p<0.05$) and the volume density of interstitium increased ($p<0.05$) after gonadectomy (Table 1); but like the control group, the stereological parameters showed no statistically significant differences between the peripheral and central regions.

The volume densities of the follicles, colloid and epithelium of the whole thyroid gland decreased in Orx animals in comparison to the SO controls by 35.5%, 28.5% and 7%, respectively ($p<0.05$). The volume density of the interstitium of the whole thyroid gland was higher in Orx dogs when compared to SO males by 35.5% ($p<0.05$).

In Orx TP group, the light and electron microscopic features of both peripheral and central thyroid follicles were exactly similar to those of the control group except for the apical microvilli and lateral and basal infoldings of the cells which showed characteristics like those of the Orx group. This means that all the morphologic changes resulted from orchidectomy in dogs seems to be reversible except those related to the cell membrane (Figure 2c).

Micrometric results showed that androgen treatment of Orx group reversed all the morphometric changes seen in Orx group. The volume densities of the follicles, colloid and epithelium in both peripheral and central thyroid regions were higher than in the Orx group ($p<0.05$). The volume density of interstitium in both peripheral and central thyroid regions was lower than in Orx group. No statistically significant differences were detected in these stereological parameters between the peripheral and central regions ($p<0.05$).

In the Orx TP dogs, the volume densities of the follicles, colloid and epithelium of the whole thyroid gland increased by 36%, 29.5% and 6.5%, respectively ($p<0.05$) in comparison to Orx dogs. The volume densities of the interstitium of the whole thyroid gland decreased by 36% ($p<0.05$) compared to Orx group.

None of the examined morphometric parameters in total thyroid tissue (in both peripheral and central thyroid zones) of Orx TP dogs were significantly changed compared with the SO group.

DISCUSSION

The follicles of thyroid gland, as defined as spheres, are the basic endocrine units which are composed of hormone-secreting cells. In some animals, the size distribution pattern of thyroid follicles is different between the peripheral and central gland regions. In rats, the larger thyroid gland follicles are mostly located at the periphery of the thyroid lobes [15]. Sekuliæ *et al*. (2007) stated that the peripheral thyroid follicles in the pigs show increased synthetic and secretory activity in comparison to the centrally placed ones [16]. Results obtained from the present study revealed that in dogs unlike rats and pigs, no statistically significant differences were detected in the stereological parameters between the peripheral and central thyroid regions.

After orchidectomy, the relative volume densities of the follicles, colloid and epithelium of the whole thyroid gland were decreased (by 35.5%, 28.5% and 7%, respectively, $p<0.05$) and that of the interstitium was increased (by 35.5%, $p<0.05$) compared to SO dogs. Chronic TP treatement of Orx dogs reversed these changes to the pre-orchidectomy state. These observed structural changes suggest that the orchidectomy induced decreased synthesis and secretion of thyroid hormones and the androgen administration reversed it.

At electron microscopic level, both the peripheral and central thyroid follicles in the Orx group showed signs of decreased activity such as decreased number of apical microvilli and lateral and basal cell interdigitations, mitochondria and free ribosomes. In Orx TP group, microscopic features of both peripheral and central thyroid follicles were exactly similar to those of the control group except for the apical microvilli and lateral and basal infoldings of the cells which showed characteristics like those of the Orx group. This means that all the morphologic changes resulted from orchidectomy in dogs seems to be reversible except those related to the cell membrane.

The observed changes of thyroid structure and ultrastructure after orchidectomy and androgen administration could be due to the direct action of male gonadal steroids on thyroid follicular cells. Thyroid gland is one of the non-classical target organs for sex steroids and presence of androgen and estrogen receptors in the pituitary thyrotrophs and neoplastic and non-neoplastic thyroid glands of mammalian species is well documented [5, 7-9, 17, 18]. Results obtained from studies regarding the thyroid-gonad relationship are variable. Gonadectomy suppresses the function of the thyroid gland in the lizards [19] and rabbits [20]; but in the rat [15] pigeon and the duck the thyroid gland is stimulated after castration and the activation of the pituitary thyrotroph shows an apparent relationship between thyroid and gonadal function [21]. Histological, morphometrical and ultrastructural results of the present study show that orchidectomy suppresses the function of the thyroid gland in male dogs.

The structural and ultrastructural changes in thyroid tissue detected in the present study, might also be contributed by the effect of TSH on the thyroid gland. TSH is a major regulator of the thyroid gland morphology, as it affects a wide variety of aspects of thyroid function. TSH is responsible for the morphological appearance of thyroid follicles and the synthesis and secretion of thyroid hormones [22]. The TSH stimulative effect on thyroid follicular cell function is modulated by the action of various molecules such as neuropeptides, peptides derived from parafollicular cells and growth factors [23]. Our finding of decreased number of major cytoplasmic organelles after orchidectomy may points to decreased stimulation of follicular cells with TSH. The clear sign of this stimulation at the light microscopic level was a decrease in the follicular epithelium height and a reduction in the colloid mass, which was supported by the stereological quantification results. The stimulatory effect of testosterone on the expression of TSH mRNA in the pituitary in the normal Wistar rats is known and in adult male rats, testosterone induces a dose-dependent change in TSH-binding to thyrocytes [17]. So the stimulatory effect of TP treatement seen in the present study may be due to the effect of this steroidal hormone on the TSH mRNA expression in the pituitary gland in addition to its direct effect on the gland.

It was concluded that gonadectomy has a partially reversible inhibitory effect on thyroid gland on male dogs.

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