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Effect of high concentration of testosterone enanthate on histometrical structure of the adrenal cortex in male rats

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Summary

Testosterone enanthate (TE) is used by many athletes as a muscle builder. Previous studies showed that high concentration of the drug in plasma may affect the natural function of endocrine glands, specially the secretion of the adrenocortical hormones. The objective of the present study was to examine the effects of high concentration of TE on histometrical structure of adrenal cortex in male rats. 120 Charles River rats were equally divided into 5 groups of gonadectomized rats receiving (G+TE), non-gonadectomized rats receiving TE (Sh+TE), gonadectomized rats receiving vehicle (G+S), non-gonadectomized rats receiving vehicle (Sh+S), and the control rats which were neither operated nor received vehicle or drug (CO). The animals were given either 0.5 ml normal saline as vehicle or 5 mg/100 g body weight TE in equal volume of normal saline. Nine weeks after receiving drug or vehicle, serum levels of testosterone were determined, and histometrical studies were performed on tissues from adrenal glands using haematoxylin and eosin staining. There was a significant increase in serum levels of testosterone in G+TE group compared to the CO one. Moreover, the diameters of the nuclei and cells from zona glomerulosa, zona fasciculate and zona reticularis showed a significant increase compared to the CO group. The results indicated that TE has an increasing effect on the nuclei and cell size of the adrenal cortex.

Key words: Testosterone enanthate, Adrenal gland cortex, Histometric, Rat

Introduction

The use of steroid drugs among youths, especially athletes, has been increasing primarily for building muscle and increasing body strength. These drugs have androgenic as well as anabolic effects on the body. The anabolic effects of the drugs have led to their misuse by individuals trying to win sport competitions. The use of anabolic drugs by weight lifting and body building athletes started in 1950s. These drugs have a significant effect on the body weight and the volume of muscle mass. It has been concluded that at supra-physiological doses, androgens can increase body strength. This hypothesis is based on the concept of different anabolic and androgenic effects of such hormones (Bhasin *et al*., 1997).

Eagerness to misuse of anabolic steroids has significantly been increased in recent years. The misuse of these drugs is not limited to athletes and many men and women use such drugs to gain favorable physical appearances (Brower *et al*., 1994).

Testosterone administrations in replacement doses to hypogonadal men and at supra-physiological doses to healthy eugonadal men were associated with significant increases in fat-free mass and muscle size (Bhasin *et al*., 1997). Similarly, testosterone replacement in immunodeficiency virus-infected men experiencing weight loss and low serum testosterone

levels induced significant gains in lean body masses and muscle volumes (Bhasin *et al*., 1998). It has recently been demonstrated that changes in circulating levels of testosterone, induced by combined administration of gonadotropin-releasing hormone agonist and graded doses of testosterone, were associated with dose- and concentration-dependent changes in fat-free mass and muscle size (Bhasin *et al*., 2001).

Androgens have significant effects on protein anabolism, water and ions retention and other metabolic processes (Katzung, 2001). There are also some reports indicating the transport of biogenic amines by these drugs (Nixon *et al*., 1979). Therefore, it could be possible that sex steroids, which have receptors in different parts of the brain, are able to affect the serum levels of pituitary hormones through different axes. Sex hormones are produced much more in sex organs, and to a lesser extent in adrenal cortex (Junqueira and Carneiro, 2003). Steroid hormones such as testosterone act by inducing protein synthesis via affecting gene expression, transcription and translation in different proteins of cell (Lodish *et al*., 2000). The objective of this study was to determine the effects of testosterone enanthate (TE) on the size of nuclei and cells of the adrenal cortex.

Materials and Methods

Adult (60-day-old) male Charles River rats weighing 200–220 g were obtained from animal breeding center, Shiraz University of Medical Sciences, Shiraz, Iran. They were kept at 25 ± 2°C and 12/12 hr light and dark with food and water *ad libitum*.

One-hundred and twenty rats were anaesthetized by intraperitoneal injection of 100 mg/kg of ketamine (Udayakumar *et al*., 1998). They were placed supine on a surgical board, and their testes were pulled out through a longitudinal midline incision in the scrotum. The incisions were then cleansed and sutured. One week later, the animals were divided equally into the following groups. Group 1 was gonadectomized rats receiving 5 mg/100 g body weight of TE (G+TE); group 2 was non-gonadectomized rats receiving 5 mg/100 g body weight of TE (Sh+TE); group 3 was gonadectomized rats receiving 0.5 ml of normal saline as vehicle (G+S); group 4 was non-gonadectomized rats receiving 0.5 ml of vehicle (Sh+S); and group 5 was the control rats which were neither operated nor received vehicle or TE (CO).

TE was administered intraperitoneally once a week for 9 weeks. At the end of the 9th week, blood samples were collected for the measurement of serum levels of testosterone using ELISA method. Animals were then sacrificed, and their adrenal glands were removed for histometrical studies using haematoxylin and eosin staining. Histometrical measurements were performed under a light microscope equipped with a micrometer eyepiece.

Data were presented as mean ± SD and analysed using one-way analysis of variance (ANOVA) by SPSS. Duncan's multiple range test was used as the *post hoc* test. A $p<0.05$ was considered statistically significant.

Results

The serum levels of testosterone in G+TE and Sh+TE groups were significantly ($P<0.05$) higher than that in the CO group. Moreover, serum levels of testosterone in G+S group was significantly lower than those in G+TE and Sh+TE groups. However, it was not significantly different as compared to CO or Sh+S groups (Fig. 1).

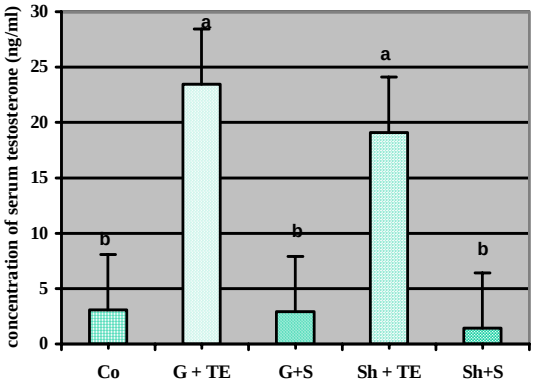

Fig. 1: Serum levels of testosterone of sham-operated rats receiving testosterone enanthate (Sh+TE) or vehicle (Sh+S), gonadectomized rats receiving vehicle (G+S) or testosterone enanthate (G+TE) and control rats (CO) on 63rd day after injection (n = 6). The groups that indicated by the same letter have no significant difference (P>0.05).

The diameters of nuclei and ZG layer cells of G+TE group were significantly higher than those of other groups. The ratio of the diameters of nuclei to the diameters of ZG layer cells from gonadectomized groups were not significantly different from each other, or from that of CO group (Table 1).

The diameter of nuclei of ZF layer cells of G+TE and Sh+TE groups were significantly ($P<0.05$) higher than those of CO group. However, the diameter of nuclei of ZF layer cells from Sh+S or G+S groups were not significantly different from that of CO group. The diameters of ZF layer cells in groups receiving TE or vehicle were not significantly different from those of CO group. The ratio of the diameters of nuclei to the diameters of ZF layer cells in Sh+S and G+S groups were significantly ($P<0.05$) higher than those from CO group (Table 2).

The diameters of nuclei of ZR layer cells from G+TE group were significantly higher than that from CO and other groups. However, the diameters of ZR layer cells of G+TE were not significantly different from those of CO group. The proportion of the diameters of nuclei to the diameter of ZR layer cells in groups receiving TE or vehicle were not significantly different from those of CO group (Table 3)

Discussion

The findings of the present study show that in the absence of gonads androgens, the adrenal glands did produce testosterone comparable to that found in the control rats. Moreover, it showed that supra-physiologic blood levels of testosterone were associated with increased diameters of ZG, ZF and ZR cells as well as the diameters of their nuclei. The testes are believed to be the main androgen-producing organ in male animals and human. The removal of such organs is expected to result in significant reduction of testosterone blood levels. However, in contrary to the expectation, removal of testes did not lead to reduction of blood levels of testosterone. This indicates that another organ might have taken over the synthesis of androgens. The most likely candidate might be adrenal gland (Isaacson *et al*., 1993). This speculation might receive support from a previous paper demonstrating that in the first few weeks after gonadectomy the blood levels of androstenedione—an androgen shown to release from adrenal glands—had been increased. Moreover, 30 days after gonadectomy, the plasma concentration of androstenedione and testosterone increased

Table 1: The mean ± SD (n = 24) of the diameter of nucleus of the ZG layer cells, the diameter of the ZG layer cells and the ratio of the nucleus of the ZG layer cells to the diameter of the ZR layer cells of sham-operated rats receiving testosterone enanthate (Sh+TE) or vehicle (Sh+S), gonadectomized rats receiving vehicle (G+S) or testosterone enanthate (G+TE) and control rats

Groups	Number of Samples	The ratio of diameters of nuclei of the ZG cells to the diameters of the ZG cells	Diameter of the ZG layer cells (μM)	Diameter of nuclei of the ZG layer cells (μM)
Co	25	0.472 ± 0.44	11.00 ± 0.32	5.15 ± 0.13
G+TE	45	0.445 ± 0.42	12.76 ± 0.33*	5.55 ± 0.10*
G+S	31	0.439 ± 0.41	11.25 ± 0.38	4.88 ± 0.14
Sh+TE	30	0.442 ± 0.41	11.00 ± 0.35	4.76 ± 0.11*
Sh+S	25	0.428 ± 0.39	11.08 ± 0.35	4.66 ± 0.11*

*Significantly ($P<0.05$) different from the control group

Table 2: The mean ± SD of the diameter of nucleus of the ZF layer cells, the diameter of the ZF layer cells and the ratio of the nucleus of the ZF layer cells to the diameter of the ZF layer cells of sham-operated rats receiving testosterone enanthate (Sh+TE) or vehicle (Sh+S), gonadectomized rats receiving vehicle (G+S) or testosterone enanthate (G+TE) and control rats

Groups	Number of samples	The ratio of the diameters of nuclei of ZF cells to the diameter of the ZF cells	Diameter of the ZF layer cells (μM)	Diameter of nuclei of the ZF layer cells (μM)
Co	25	0.360 ± 0.34	14.12 ± 0.29	5.04 ± 0.81
G+TE	46	0.438 ± 0.41*	13.40 ± 0.84	5.78 ± 0.56*
G+S	36	0.383 ± 0.35	13.61 ± 0.78	5.09 ± 0.88
Sh+TE	30	0.415 ± 0.39*	12.74 ± 0.06	5.25 ± 0.01
Sh+S	25	0.389 ± 0.36	13.54 ± 0.34	5.13 ± 0.88

*Significantly ($P<0.05$) different from the control group

Table 3: The mean ± SD of the diameter of nucleus of the ZR layer cells, the diameter of the ZR layer cells and the ratio of the nucleus of the ZR layer cells to the diameter of the ZR layer cells of sham-operated rats receiving testosterone enanthate (Sh+TE) or vehicle (Sh+S), gonadectomized rats receiving vehicle (G+S) or testosterone enanthate (G+TE) and control rats

Groups	Number of samples	The ratio of the diameters of nuclei of ZR cells to the diameters of ZR cells	Diameter of ZR layer cells (μM)	Diameter of nuclei of ZR layer cells (μM)
Co	22	0.38 ± 0.36	13.27 ± 0.42	5.09 ± 0.11
G+TE	44	0.42 ± 0.40	13.47 ± 0.33	5.63 ± 0.11*
G+S	32	0.41 ± 0.39	12.03 ± 0.32*	4.87 ± 0.10
Sh+TE	30	0.41 ± 0.38	12.00 ± 0.37*	4.85 ± 0.12
Sh+S	22	0.42 ± 0.39*	12.17 ± 0.39	5.10 ± 0.15

*Significantly ($P<0.05$) different from the control group

in male rats (Canonaco *et al*., 1989).

This study also showed that long-term supra-physiologic blood levels of testosterone increases the diameter of ZG layer cells, and the diameter of their nuclei, while the ratio of nuclei diameter to the diameter of ZG layer cells remained unchanged. These changes indicate that ZG layer cells were likely hypertrophied involving both nucleus and cytoplasm. However, our findings did not agree with previous reports revealing no differences in the proportion of nuclei diameter to the diameter of ZG layer cells of adrenal cortex in male and female rats as well as in gonadectomized rats receiving gonadal hormones (Malendowicz *et al*., 1986). Moreover, gonadectomy and testosterone therapy were shown not to affect the mitotic index of ZG layer cells (Malendowicz *et al*., 1986). Therefore, the effects of androgens and estrogens on steroidogenic activity of the adrenal cortex in association with protein synthesis may not be ignored (Nowak *et al*., 1995).

Histometrical studies also showed that the diameter of ZF cells as well as the diameter of their nuclei and the ratio of the diameter of nuclei to the diameter of ZF layer cells did increase in gonadectomized rats receiving testosterone. It also demonstrated that the nuclei diameter of ZR cells increased in such rats. The increase in the size of cells from adrenal cortex indicated adrenocortical hypertrophy of the adrenal cortex, which is in agreement with a previous report (Leodolter *et al*., 1973). These results might indicate more activity of nucleus and rough endoplasmic reticulum in long-term in association with transcription and protein synthesis. Such proteins have significant effect on the function of smooth endoplasmic reticulum which is responsible for synthesis of steroid hormones (Lodish *et al*., 2000).

In conclusion, our findings indicate that gonadectomized rats did have serum levels of testosterone comparable to that of non-gonadectomized rats. The most likely organ to produce testosterone in these animals is adrenal gland. The study also showed that administration of a supra-physiologic dose of TE was associated with the hypertrophy of adrenal cortex (Kumar *et al*., 2003).

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