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Androgen Receptor in Rat Adrenal Gland after Treatment with Anabolic Androgenic Steroids and Submaximal Training

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The anabolic androgen steroids (AAS) find wide application in contemporary medicine and in sport. Their influence on the morphology and function of the adrenal gland are target of numerous studies. There is no information on the combined influence of AAS and continuous endurance training on different organs. The purpose of the present study is to follow the influence of AAS and submaximal training on the function of the adrenal gland by immunohistochemical expression of the androgen receptors in the adrenal cells. Male white Wistar rats divided into two groups are used. One of the groups exercised on treadmill with sub maximal loading (65-70%VO_2max) 5 day/wk for 8 weeks and was simultaneously treated with 10mg/kg Nandrolone Decanoate (ND) i.m. The other group did not exercise and were treated with Placebo (Pl) i.m. After eight weeks the animals were decapitated and the adrenal glands were extracted for further study. The androgen receptors were proven immunohistochemically by the avidin-biotin-peroxidase complex (ABC) method was applied using Vectastain® *Elite* ABC kit (Vector, USA). Immunopositive nuclei were observed in the cortex cells of both groups of animals, but without positive reaction in the medulla cells. In the animals of the T+ND group a significant decrease of the intensity of the immunocytochemical reaction was observed. Consequently the simultaneous application of AAS and endurance training apparently results in decreasing of the AR immonohistochemical activity.

Key words: rat adrenal cortex, androgen receptor, anabolic androgenic steroids, submaximal training.

Introduction

The use of androgenic anabolic steroids (AAS) by sportsmen and non-athletes is increasing. The continuous acceptance of high doses AAS combined with physical training results in psychiatric and physiologic changes in the organism. A lot of authors study the effects of AAS on hypothalamic-pituitary-adrenal axis function and more accurately on the morphology and function of the adrenal cortex cells [13]. The changes in the steroidogenesis in the adrenal cortex of rats after a prolonged treatment with AAS are histochemicaly proven in our previous studies [11]. The influence of androgens on growth, differentiation and function of different mamma-

lian tissues is realized by a specific intracellular receptor and modification of gene expression [10]. Androgen receptor (AR) has been identified in the adrenal cortex of immature and adult Rhesus monkeys by immunocytochemistry [9] and in adrenocortical tissue of adult rat by immunoblot assay [1]. Such data has been reported in other steroidproducing cells like the Leydig cells of rat testis [6, 7]. On the other hand, a lot of authors reported functional changes in the adrenal gland, predominantly in the adrenal medulla under different physical exercises and stress conditions [5] but there are less studies on the cortex cells.

Our study is a part of a project for investigation of the influence of AAS on endurance performance in different tissues and organs. The aim of the present work was to investigate the immunohistochemical expression of androgen receptors in rat adrenal cortex after simultaneous treatment with anabolic androgenic steroids and submaximal training.

Material and Methods

Male Wistar rats were distributed into 2 groups. One of them exercised on treadmill with submaximal loading (65-70% VO_2 max) 5 day/wk for 8 weeks. After 2 weeks all of the trained and sedentary rats received weekly either 10 mg/kg — Nandrolone Decanoate (ND) or Placebo (P) i.m. for 6 weeks. Day after the last exercises all the groups: 1. sedentary + Pl (S+P) and 2. trained + ND (T+ND) were decapitated. Adrenal cortex fragments were fixed in Bouin's fluid for 24 hours and embedded in paraffin. For the antigen detection the avidin-biotin-peroxidase complex (ABC) method was applied using Vectastain® *Elite* ABC kit (Vector, USA) using primary polyclonal rabbit anti-androgen receptor antibody (Alpha Diagnostics; 1:100). The peroxidase activity was then developed by means of the Peroxidase Substrate kit (DAB), (Vector, USA). *As controls*, sections were used in which the primary or secondary antibodies were replaced by phosphate-buffered saline (PBS) or only the peroxidase activity was visualized.

Results

Our results demonstrated that there was positive immnohistochemical expression of AR in the rat adrenal gland of both examined groups. In the animals of the S+P group immunocytochemistry revealed that the staining for AR was located in the cell nuclei. AR reaction was more prominent in the cells of zona fasciculata and zona reticularis compared with the zona glomerulosa. No positive signals were detected in the medulla cells (Fig.1). In the animals of the T+ND group a significant decrease of the intensity of the immunocytochemical reaction was observed with the same nuclear localization of AR. The staining was again lower in the cells of the zona glomerulosa than both other zones of adrenal cortex (Fig. 2).

Discussion

AR is a member of the steroid-thyroid hormone-retinoic acid family of transcription regulatory factors [14]. Understanding the regulation of the expression of these receptors is fundamental for elucidating the mechanism of various hormones' action. It is a well-documented fact that AR was also localized in the nuclei of the adrenal cortical cells [4]. It is known that adrenal androgens are secreted by zona

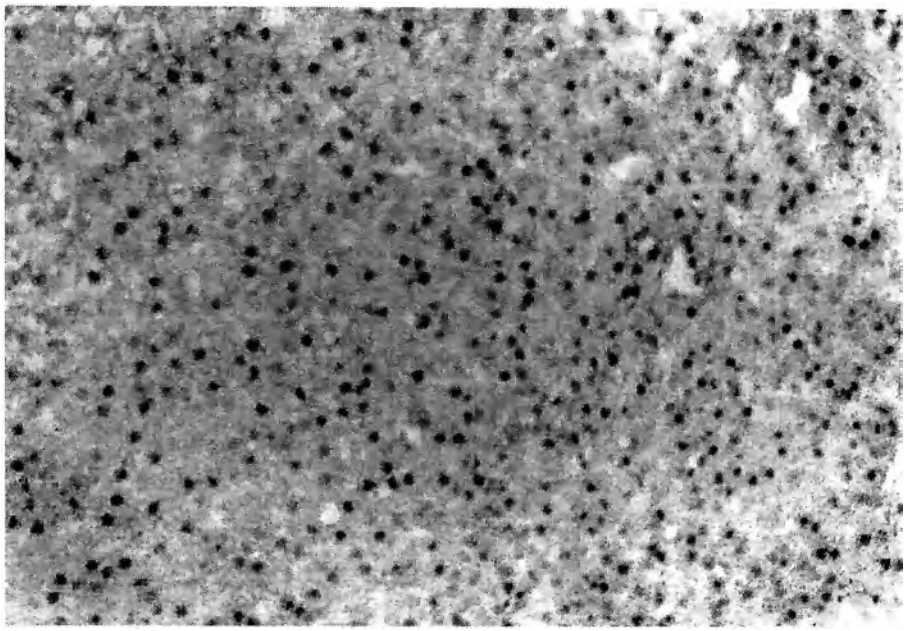

Fig. 1 Immunohistochemical expression of AR in adrenal cortex cells — S+P group (× 100)

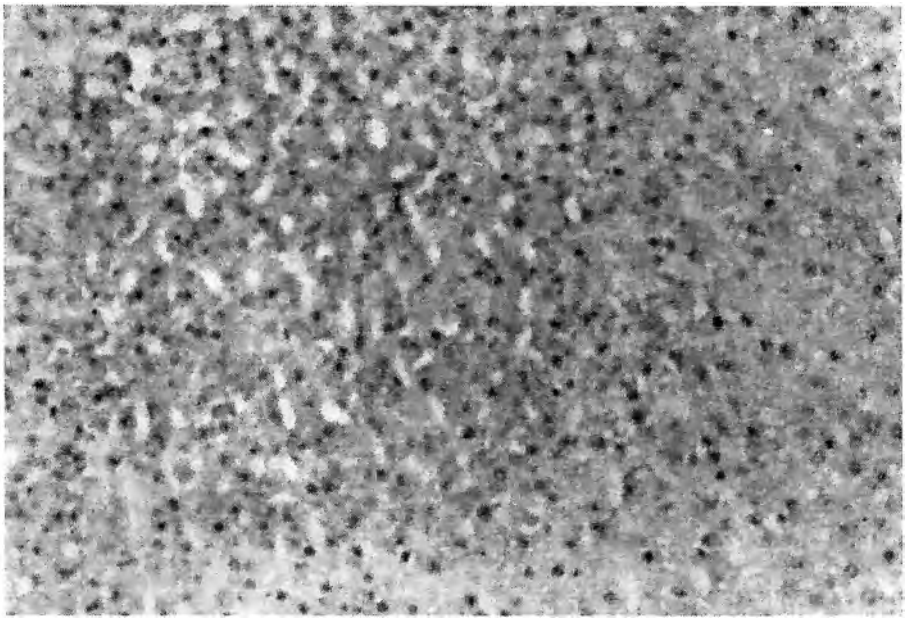

Fig. 2 Immunohistochemical expression of AR in adrenal cortex cells — T+ND group (× 100)

reticularis. The presence of staining of cell nuclei of the three zones suggests that AR may be involved in the autocrine function. The weaker AR expression in the T+ND group of our study suggest that adrenal cortex function is decreased, which is connected to the continuous treatment with ND combined with submaximal training. These facts show that AAS has influence on the functional activity of the adrenal cortex [9]. Long-term administration of AAS leads to adrenal insufficiency [2]. The changes in the functional activity of the adrenal cortex cells under physical exercises are very disputable. They depend on the continuation as well as on the load of the physical training. Changes in the secretion of cortisol and adrenocorticotropin hormone are found out [3]. There are no data about the effect of endurance training on the adrenal cortex morphology as well as the combination of endurance training and AAS. Our previous results showed changes in the immunohistochemical expression of AR in other steroidproducing cells like the rat Leydig cells following AAS administration and endurance training [8].

In conclusion, the combination of both factors — long-term AAS treatment and submaximal training apparently results in decreasing of the AR immonohistochemical activity in adrenal cortex cells. This might be one of the modes of action of AAS and physical training on the functional activity of the rat adrenal cortex.

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