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Combined effects of androgen anabolic steroids and physical activity on the hypothalamic–pituitary–gonadal axis

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ABSTRACT

Analysing effects of pharmaceutical substances and training on feedback mechanisms of the hypothalamic–pituitary–gonadal axis may be helpful to quantify the benefit of strategies preventing loss of muscle mass, and in the fight against doping. In this study we analysed combined effects of anabolic steroids and training on the hypothalamic–pituitary–gonadal axis. Therefore intact male Wistar rats were dose-dependently treated with metandienone, estradienedione and the selective androgen receptor modulator (SARM) S-1. In serum cortisol, testosterone, 17β-estradiol (E2), prolactin, inhibin B, follicle-stimulating hormone (FSH), luteinizing hormone (LH), Insulin-like growth factor 1 (IGF-1), and thyroxine (T4) concentrations were determined. Six human volunteers were single treated with 1-androstenedione. In addition abusing and clean body builders were analysed. Serum concentrations of inhibin B, IGF-1, cortisol, prolactin, T4, thyroid-stimulating hormone (TSH), testosterone and LH were determined. In rats, administration of metandienone, estradienedione and S-1 resulted in an increase of muscle fiber diameter. Metandienone and estradienedione but not S-1 administration significantly decreases LH and inhibin B serum concentration. Administration of estradienedione resulted in an increase of E2 and S-1 in an increase of cortisol. Single administration of 1-androstenedione in humans decreased cortisol and inhibin B serum concentrations. LH was not affected. In abusing body builders a significantly decrease of LH, TSH and inhibin B and an increase of prolactin, IGF-1 and T4 was detected. In clean body builders only T4 and TSH were affected.

1. Introduction

Age- and disease-dependent loss of muscle mass (muscle wasting) has detrimental consequences for human health, by causing metabolic disturbances and physical frailty in patients. Consequently, muscle wasting is associated with an increased risk of developing metabolic diseases, such as type-2 diabetes or the metabolic syndrome [1].

There are numerous reasons for the loss of muscle mass, which may justify the usage of the term 'muscle wasting syndrome'. Disease-associated muscle wasting (cachexia) develops as a consequence of several common diseases, such as cancer, AIDS, heart failure, chronic obstructive pulmonary disease (COPD) and renal failure. Patients suffering from these diseases and developing cachexia have to face increased incidence of morbidity and mortality [2]. Another major reason for muscle wasting is muscle disuse due to

Abbreviations: 1-AD, 1-androstenedione; AIDS, acquired immune deficiency syndrome; AR, androgen receptor; AS, anabolic steroid; COPD, chronic obstructive pulmonary diseas; E2, 17β-estradiol; ELISA, enzyme linked immunosorbent assay; ERE, estrogen response element; Estra, estradienedione; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HPGa, hypothalamic–pituitary–gonadal axis; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; Meta, metandienone; SARM, selective androgen receptor modulator; T3, triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

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a spreading sedentary life style [3]. A scenario of extremely high relevance for the health status of the population is the gradual decrease in the ability to maintain skeletal muscle function and mass during aging (sarcopenia). For these reasons there is an urgent need to develop strategies for prevention or treatment of skeletal muscle loss. Increase or maintenance of muscle mass includes de novo protein synthesis and the activity of satellite cells (muscle stem cells). De novo protein synthesis is believed to be the key mechanism in hypertrophic muscle adaptation in response to strength training [4]. Nutrition, physical exercise but also endocrine stimuli have been demonstrated to increase protein synthesis rates [5–7]. A loss of protein synthesis has been shown in some cases of muscle wasting especially cancer cachexia [8].

Satellite cells are the basis for skeletal muscle recovery and regeneration following injury caused by trauma or unfamiliar exercise. In a rat model Schmalenbruch and Lewis could demonstrate that unloaded adult muscle has a relatively slow nuclei turnover of 1–2% compared to young muscle [9]. This is known to change dramatically in response to injury. Satellite cells are activated from their quiescent state, proliferate, and contribute to skeletal muscle regeneration by either fusing to the damaged myofibers (hypertrophy) or producing new myofibers (hyperplasia) [10].

The activity of satellite cells is controlled by a variety of factors like hormones and growth factors, but also by the immune system. The inability to activate satellite cells after injury is discussed as a major mechanism responsible for age dependent loss of muscle mass [11]. However, physical activity, particularly strength training is able to stimulate satellite cell activity even in the elderly [12,13]. The individual reasons for the development of sarcopenia and cachexia are diverse and multifactorial. Decreased synthesis of different anabolic hormones (testosterone, estradiol) or growth factors like insulin-like growth factor-1 (IGF-1), and increasing concentrations of negative growth factors like myostatin together with accelerated catabolism and decreased anabolic activity lead to reduced satellite cell activation and de novo protein synthesis.

Therefore strategies for preventing the loss of skeletal muscle mass include pharmacological interventions, training concepts or combinations. However, to develop individualized intervention concepts and to quantify the effects of such concepts, techniques to detect the individual anabolic response are needed.

A classical pharmacological strategy to induce anabolic effects is the administration of testosterone or synthetic derivates like anabolic steroids (AS) or selective androgen receptor modulators (SARMs). From the classical anabolic steroids, including testosterone, it is known that exogenous administration of these substances directly affects the production of endogenous hormones via feedback mechanisms on the hypothalamic–pituitary–gonadal axis (HPGa) [14]. This concept is very efficient why treatment with low testosterone doses is a well established pharmaceutical concept for male contraception [15,16]. The HPGa is regulated by complex androgenic negative feedback mechanisms like decreased GnRH synthesis in the hypothalamus, decreased gonadotropin secretion in the pituitary gland, as well as a non-steroidal feedback of inhibin B on FSH secretion in the pituitary gland [17]. Therefore the simultaneous detection of hormones involved in this regulation could be a promising strategy to detect and quantify the anabolic response of an individual to a pharmacological intervention but also to training. Moreover it will allow the detection of side effects and may be a promising tool to detect the abuse of anabolic drugs for doping purposes. Therefore it was the aim of this study to detect prolactin, cortisol, TSH, FSH, and LH in combination with inhibin B, 17β-estradiol (E2), thyroxin (T4) and testosterone levels and indicators for anabolic activity, such as IGF-1 simultaneously, to identify and quantify the anabolic response towards the administration and misuse of anabolic substances, including selective androgen receptor modulators (SARMs) and training.

2. Material and methods

2.1. Animal experiment

Male Wistar rats (200–224 g) were obtained from Janvier (Le-Genest St-Isle, France). The rats were kept under controlled conditions (temperature 20 ± 1 °C, humidity 50–80%, 12 h light/12 h dark cycle) with free access to tap water and a diet low in phytoestrogen content (R/M-H, from Ssniff GmbH, Soest, Germany). They were maintained according to the European Union guidelines for the care and use of laboratory animals. The study was reviewed and approved by the regional administration of the governmental body.

After one week of acclimatization the rats were randomly allocated in treatment groups: $n = 8$ animals per group except 5.0 S-1 group with $n = 6$ (Table 1). The animals were treated daily with metandienone, estradienedione, or S-1 dose-dependently (0.5, 1.0 or 5.0 mg kg^{-1} bw^{-1}) or vehicle for 21 days via subcutaneous (s.c.) injection. The anabolic steroids were diluted in a solution of 20% DMSO and 80% peanut oil. The used anabolic steroids were provided by the Institute of Biochemistry, German Sport University Cologne. The purity of the substances was verified by mass spectrometry. A survey about the design of the study is given in Fig. 2A.

2.1.1. Tissue collection and preparation

After treatment period and determination of the body weight, the animals were sacrificed by decapitation. Blood samples were collected and centrifuged at 4 °C. The serum was aliquoted and frozen at −20 °C for later analyses.

Following organ removal, wet weights of the prostate, seminal vesicle, liver, heart, M. levator ani, M. gastrocnemius and M. soleus were determined. For molecular analyses, the skeletal muscles were directly frozen in liquid N_2 or embedded in Tissue Tek (Sakura, Staufen, Germany), cooled down in isopentane (−70 °C) and afterwards in liquid nitrogen. They were stored at −80 °C for histological analysis.

2.1.2. Analyses of the animal serum samples

The serum concentration of LH and FSH where measured by Bayer AG, Leverkusen, Germany. Commercially available and for rats validated ELISAs where used for the determination of the serum concentration of testosterone, T4, prolactin, IGF-1 and inhibin B, (REF: DEV9911; Cat. no.: DET4105T100; REF: DEV9966; REF: DEE025 from DEMEDITEC, Germany and REF: A811303, Beckman Coulter, USA). The serum concentration of E2 and cortisol were determined by using a radioimmunoassay (REF: DSL4800 and REF: IM1841 from Beckman Coulter, USA).

Table 1
Treatment groups of the animal study. Q6

Group	n	Treatment dosage [mg kg^{-1}]
Control	8	Vehicle
0.5 Met	8	0.5 Metandienone
1.0 Met	8	1.0 Metandienone
5.0 Met	8	5.0 Metandienone
0.5 Est	8	0.5 Estradienedione
1.0 Est	8	1.0 Estradienedione
5.0 Est	8	5.0 Estradienedione
0.5 S-1	8	0.5 SARM S-1
1.0 S-1	8	1.0 SARM S-1
5.0 S-1	6	5.0 SARM S-1

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