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Tetrahydrogestrinone is a potent but unselective binding steroid and affects glucocorticoid signalling in the liver

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Abstract

Tetrahydrogestrinone (THG) is a steroid recently identified to be misused as doping agent. However, the knowledge on functions of this substance in humans or animal models is rather limited. Therefore, it was our aim to further characterize the pharmacological profile of THG and identify potential adverse side effects. THG was synthesized, the purity was confirmed and its biological activity was tested. The potency of THG to transactivate AR dependent reporter gene expression was two orders of magnitude lower compared to dihydrotestosterone. THG binds with high affinity but unselective to the androgen (AR), progesterone (PR), glucocorticoid (GR) and mineralocorticoid (MR) receptor. Treatment of orchiectomised rats with THG resulted in a stimulation of prostate, seminal vesicle and levator ani muscle, indicating androgenic and anabolic properties. In the liver THG, in contrast to testosteronepropionate (TP), down regulates the expression of the GR dependent tyrosine aminotransferase gene (TAT).

In summary, our results demonstrate that THG is not a specific AR agonist. THG exhibits a high binding affinity to all tested steroid hormone receptors and binds with highest affinity to the GR. Our in vivo data are indicative of an anabolic and androgenic potency of THG, but the repression of TAT demonstrates that THG also interferes with the glucocorticoid hormone system. Therefore, it is conceivable that an intake will result in adverse side effects.

Keywords: Tetrahydrogestrinone; Androgen; Mineralocorticoid; Glucocorticoid; Progestrone receptor; Anabolic steroid; Doping; Liver

1. Introduction

Anabolic-androgenic steroids (AAS) have been banned in sport since 1980. According to the worldwide doping control statistics they are the most frequently detected substances (WADA, 2004). Since the ban of AAS, athletes and their entourage have always tried to find new products and applications to evade the doping controls. Rumours of new steroids, produced and used specifically to escape doping tests in sport have been spread since more than 15 years. In 2002, Catlin et al. found a never-marketed steroid called norbolethone in two urine samples. In 2003, a track and field coach sent

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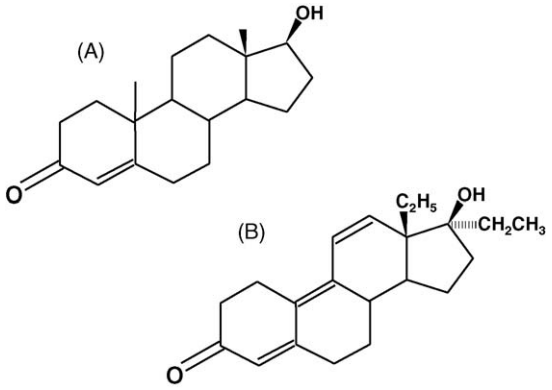

Fig. 1. Chemical structure of testosterone (A) in comparison to tetrahydrogestrinone (THG) (B).

a syringe with an oily substance to the United States Anti-Doping Agency. The substance proved to be tetrahydrogestrinone (THG, Fig. 1), the first true "designer steroid", designed, synthesized and distributed solely as an undetectable doping agent (Catlin et al., 2004). Even more recently, a new "designer steroid", desoxymethyltestosterone (DMT) or "madol" was discovered, and its detection in urine described (Sekera et al., 2005). This reflects an alarmingly sophisticated, illicit manufacturing facility and an underground network to distribute these substances. There exists an unknown number of abusers of such substances in sport, even though their anabolic-androgenic potency and hormonal properties are not yet resolved. For doping prevention and control it is essential to know the effects and side effects of these "designer steroids" to plan informational and educational campaigns for athletes.

Therefore, the aim of our study was to further characterize the pharmacological profile of THG and identify potential adverse side effects. THG was synthesized and its identity, purity and biological activity was tested. The specificity of a steroid for the binding to distinct steroid hormone receptors is a general issue with respect to potential side effects. Therefore, the binding affinity of THG to the androgen (AR) but also to the mineralocorticoid (MR), glucocorticoid (GR) and progesterone receptor (PR) was determined. To verify if the detected binding preferences to the distinct steroid hormone receptors result in a biological activity in vivo, orchiectomised rats were treated for 10 days with equimolar doses of THG and the reference compound testosteronepropionat (TP). Effects on prostate, seminal vesicle, heart and the levator ani muscle were investigated. Furthermore the regulation of the GR dependent tyrosine aminotransferase gene expression (TAT) was assessed in the liver.

2. Materials and methods

2.1. Substances

Androstendione and testosteroneproprionate were provided by the Institute of Biochemistry, German Sport University Cologne. Purity of the substances was verified by mass spectrometry. Dihydrotestosterone (DHT) was obtained by Sigma–Aldrich (Deisenhofen, Germany). R1881, Aldosterone, Dexametasone and Progesterone were provided by the Schering AG (Berlin, Germany).

2.2. Synthesis of THG

THG was prepared from 500 mg (1.6 mmol) of gestrinone, which was dissolved in 250 ml of methanol in a round-bottomed flask. 10 mg of PtO_2 were added, and hydrogen was flushed into the reaction mixture. After 25 min, the mixture was allowed to stand at room temperature for 30 min for sedimentation of the catalyst. The methanolic layer was transferred to a new flask, evaporated to dryness, and the resulting product was purified from residues of PtO_2 by the addition of 100 ml of deionized water and liquid-liquid extraction into tert-butyl methyl ether (TBME). To obtain THG, TBME was evaporated to dryness.

2.3. Yeast reporter gene assay

For the assessment of the androgenicity a concentration dependent assay in the widely used androgen inducible yeast screen androgen receptor assay was performed (Sohoni and Sumpter, 1998). The yeast based androgen receptor assay was cultured as previously described (Zierau et al., 2003). The yeast strain contained both a stably transfected androgen receptor (AR) construct and an expression plasmid carrying androgen-responsive sequences controlling the reporter gene lac-Z encoding the enzyme β-galactosidase. Androgenic activity from the enzymatic hydrolysis of chlorophenol red β-D-galactopyranoside was read at 540 nm using a colorimetric assay. In a concentration dependent analysis of reporter gene activity, the half maximal induction of β-galactosidase activity was a direct measure for the affinity of the compound to the AR, and therefore the androgenic activity could be estimated.

2.4. Receptor binding assay

Cytosols containing the relevant steroid hormone receptors were provided by Schering AG Berlin. Increasing concentrations of the investigated substances were incubated in the presence of 5 nM [^{3}H] R1881 (a synthetic AR agonist with high metabolic stability), [^{3}H]progesterone, [^{3}H]aldosterone or [^{3}H]dexamethasone for 16 h at 0–4 °C in the presence or absence of the indicated concentrations of unlabeled substances.