The so-called „designer steroids“ Norbolethone, Desoxymethyltestosterone and Tetrahydrogestrinone: Endocrine pharmacological characteristics and side effects in regard to doping.

Tsuyuki Nishino, Thorsten Schulz, Renate Oberhoffer, R. Klaus Müller, Horst, Michna†

Abstract

The anabolic androgenic steroids belong to the list of prohibited substances given by the WADA´s List Committee. In fact, not all the biological and toxicological risks especially when administered for doping purposes are unknown. Thus, we completely characterized the substances Norbolethone, Desoxymethyltestosterone (DMT) or Tetrahydrogestrinone (THG) concerning their endocrine and pharmacological properties.

Besides conceiving chemical possibilities to access the modified steroids, we characterized their hormonal potential using modified industrial methods like Hershberger and Clauberg assays. The synthesis of Norbolethone starts with Norgestrel and its nickel-catalyzed hydrogenation resulting in Norbolethone. Norbolethone caused a lot of bleeding disorders and many androgenizing side effects.

DMT is an anabolic steroid, which was synthesized and patented in 1961. The synthesis of DMT starts with epiandrosterone, a natural reduction product of testosterone that is excreted in urine. DMT is a potent anabolic compound and therefore it should be considered as a toxic drug. No (anti-)gestagenic, (anti-)estrogenic or (anti-)glucocorticoid potency could be detected.

The synthesis of THG starts with gestrinone and its nickel-catalyzed hydrogenation resulting in THG. By modifying the 17α-position, THG becomes orally active. THG is a very strong anabolic agent with an increased risk of liver damage and the incidence of general side effects usually caused by steroids. THG, like other anabolic steroids, exerts androgenic and progestational effects in the standard assays to predict activity in humans.

Norbolethone and DMT exert mainly androgen-anabolic effects, whereupon THG was identified to additionally induce progestational modulations.

Therefore, these compounds may induce tremendous biomedical side effects and must be considered as perilous drugs, especially when used in high doses for doping purposes.

1. Introduction

The so-called designer steroids (Fig. 1) were detected in a still growing list of Olympic record holders and prestigious athletes. Many of the top athletes were customers of BALCO laboratories, Bay Area Laboratory Co-Operative, who produced THG on a grand scale. The compound class belong to the list of forbidden steroids prohibited by the WADA/IOC and moreover these compounds have never been marketed as a pharmaceutical. In fact, the biological and toxicological activities are unknown. Within this issue, in 2003 the federal drug adminstration (FDA) declared that THG is an unapproved new drug, which cannot be legally marketed under the agency´s rigorous approval standards because this includes that drugs that are sold to American consumers are safe and effective. Thus, we set out to completely endocrine pharmacologically characterize the three steroids.

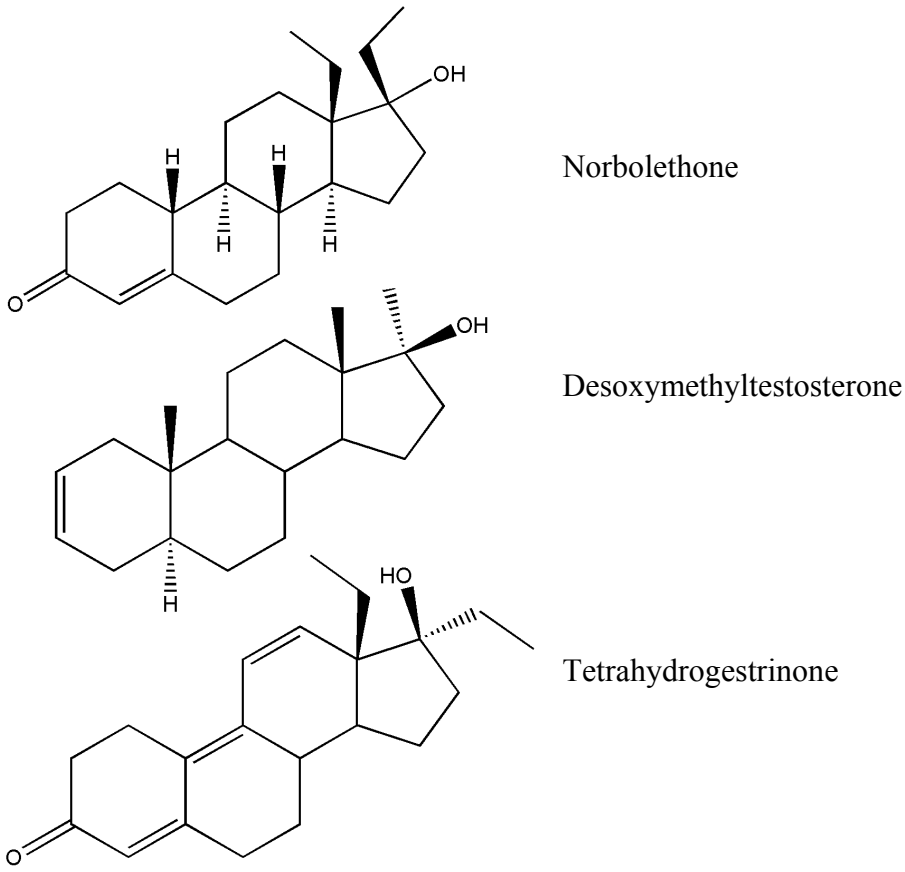

Fig. 1: Designer steroids: Norbolethone, Desoxymethyltestosterone and Tetrahydrogestrinone

2. Methods

Besides conceiving various chemical possibilities to access structurally modified steroids like Norbolethone, Desoxymethyltestosterone (DMT) or Tetrahydrogestrinone (THG), we characterized their hormonal potential using *in vitro* methods like **C**hloramphenicol **A**cetyl **T**ransferase (CAT) and **R**elative **B**inding **A**ffinity (RBA) assays and modified industrial standard *in vivo* methods like Hershberger and Clauberg assays [1,2].

Norbolethone: The synthesis of Norbolethone (Fig. 2), for example, starts with Norgestrel and its nickel-catalyzed hydrogenation (17α-alkine) resulting in Norbolethone. Norgestrel is a totally synthetic progestational agent frequently used in certain birth control pills. There are various chemical possibilities to access structurally modified steroids like Norbolethone beginning with its total synthesis. Originally developed by a US pharmaceutical company in 1966, the project was internally abandoned a few years later because of unacceptable –not published– adverse side effects. Norbolethone is mainly toxic, caused a lot of bleeding disorders and many androgenizing side effects [3].

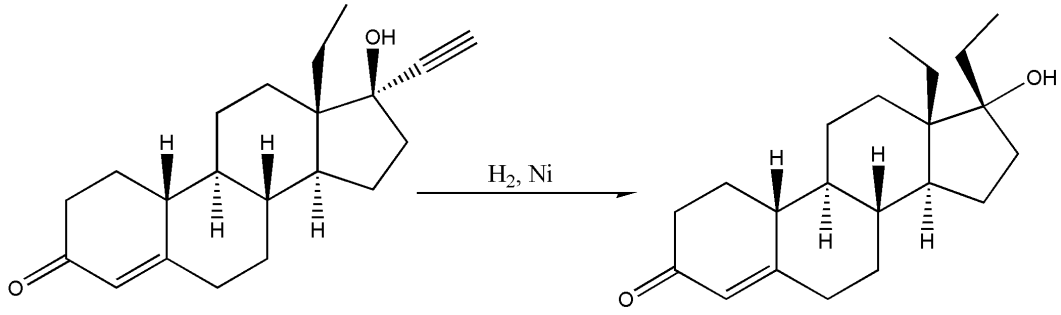

Fig. 2: Nickel-catalyzed hydrogenation of Norgestrel resulting in Norbolethone

Desoxymethyltestosterone: DMT (17α-methyl-5α-androst-2-ene-17β-ol) is an anabolic steroid, which was initially synthesized and patented in 1961 [4,5,6]. The synthesis of DMT (Fig. 3) starts with epiandrosterone, a natural reduction product of testosterone that is excreted in urine. Epiandrosterone will be reacted with p-toluenesulfonyl chloride and trimethylpyridine to remove the hydroxyl group at C-3 of the steroid ring system. After elimination of hydrochloric acid, a pair of olefin isomers form: the 3-ene and 2-ene. Reaction of these intermediates with methyllithium adds a methyl group to C-17α and converts the keto group there to a C-17β hydroxyl group, resulting in DMT and its isomer. DMT is a potent anabolic compound. The question is why it failed in further clinical trials and therefore it should be considered as a toxic drug. Anyhow, no (anti-)gestagenic, (anti-)estrogenic or (anti-)glucocorticoid potency or side-effects were published until 2006.

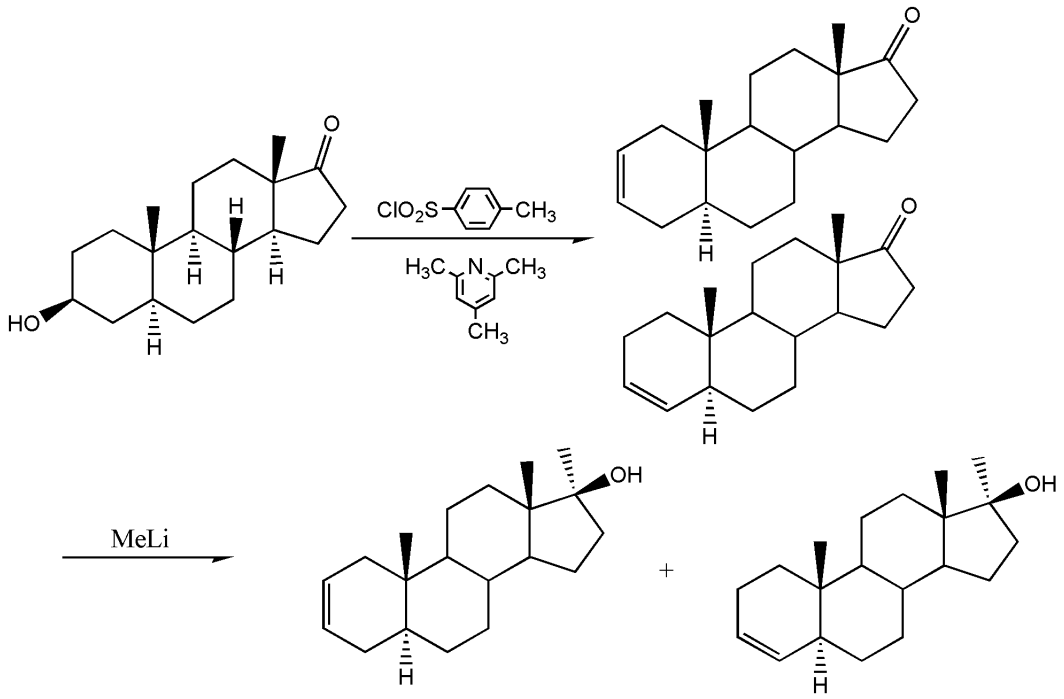

Fig. 3: Synthesis of Desoxymethyltestosterone (DMT) starting with Epiandrosterone

Tetrahydrogestrinone: The synthesis of THG (Fig. 4) starts with gestrinone and its nickel-catalyzed hydrogenation (17α-alkine) resulting in THG. By modifying the 17α-position, THG becomes orally active. THG is a very strong anabolicum with an increased risk of liver damage and the incidence of general side effects usually caused by steroids. THG, like other anabolic steroids, exerts androgenic and progestational effects in the standard assays to predict activity in humans [7].

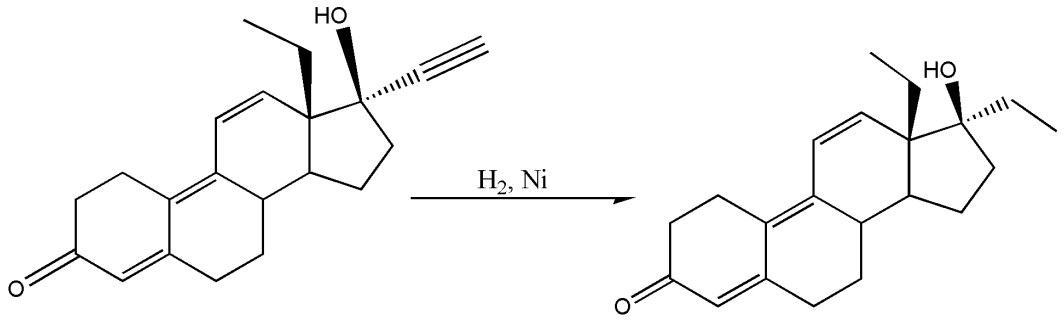

Fig. 4: Nickel-catalyzed hydrogenation of Gestrinone resulting in Tetrahydrogestrinone

3. Results

Norbolethone (Genabol) showed a low propensity for estrogenic activity or aromatization. It has weaker androgenic but more anabolic properties. This 19-nor anabolic steroid does not have a tendency to convert to estrogens and will not break down to a metabolite in androgen target tissues. There is some progestin activity with this substance similar to DMT (compare Tab. 4).

In vitro data displayed a strong binding affinity of DMT to the androgen receptor (AR): AR competition factor DMT 19, Cyproterone Acetate (CPA) 12

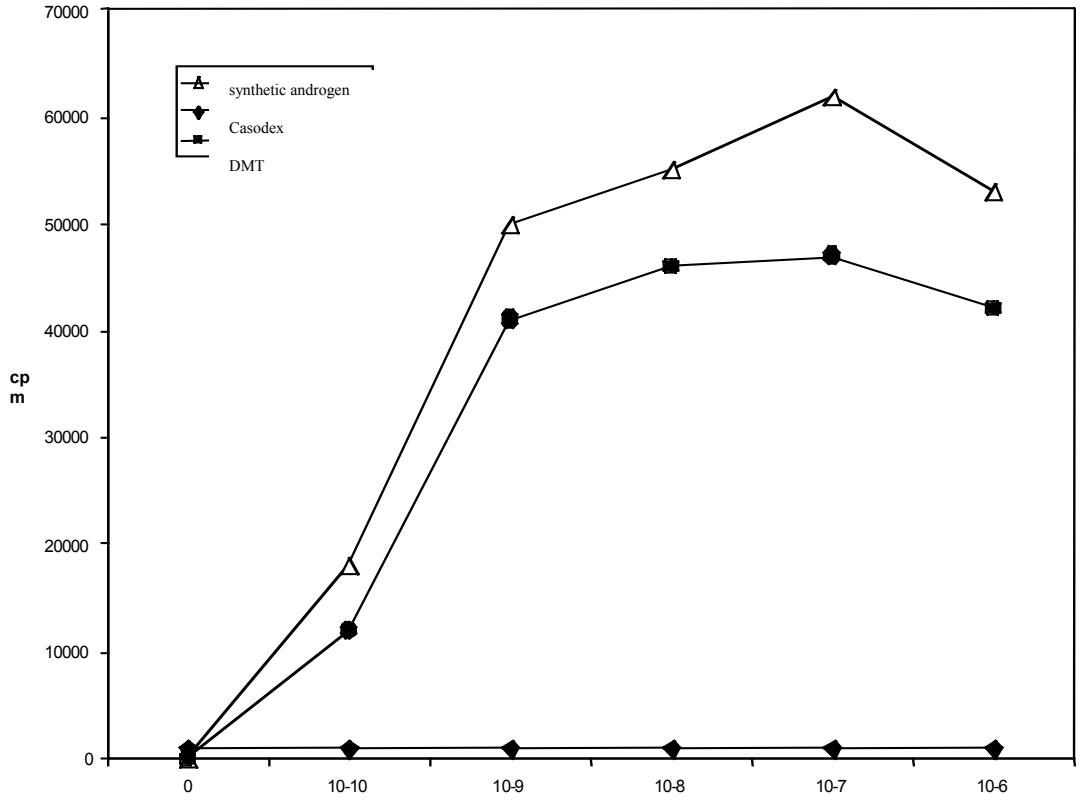

Fig. 5: Androgenic activity of DMT in CAT-Assay

and for Casodex 121. CAT assay (Fig. 5) displayed a strong androgenic activity of Desoxymethyltestosterone. The (anti-)androgenic efficaciousness of DMT was evaluated using the 12-day Hershberger Assay [8]. Orchiectomized mice and rats were treated with DMT and compared to vehicle control groups, including testosterone propionate substituted control groups. Prostates, seminal vesicles and blood samples (serum LH) were harvested after 12 days and prepared for further analysis. DMT showed a dose-dependent androgen-like effect on all parameters analyzed. Flutamide antagonized those DMT effects steadily.

Tab. 1: 12-day treatment of orchiectomized rats in Hershberger Assay. TP: Testosterone Propionate, CPA: Cyproterone Acetate; Significant compared to TP ($p<0,05$, Kruskal-Wallis-Test) #Significant compared to TP+CPA ($p<0,05$, Kruskal-Wallis-Test)

Group	Dose [mg/100g bw] (s.c.)	Organ wet weights in [mg/100g bw]	
		Seminal vesicle	Prostate
Orchiectomy	Placebo	20.0±3.0*	10.0±3.0*#
TP	0.1	116.0±23.0#	49.0±17.0#
TP+CPA	3.0+0.1	22.0±3.0*	17.0±4.0*
TP+DMT	3.0+0.1	130.0±30.0	57.0±17.0

No biologically relevant (anti-)estrogenic (Tabs. 2 and 3), (anti-)gestagenic (Tab. 4) or (anti-)glucocorticoid (data not shown) potency could be detected using the industrial standard methods for steroidal hormone potency evaluation.

Tab. 2: Influence of DMT and Estradiol on organ weights of uterus and vagina in mice and rats. *Significant compared to controls ($p<0.05$, Kruskal-Wallis-Test)

Group	Dose (s.c.)	Mice [n]	Organ weight in [mg]/10g bw		Rats [n]	Organ weight, [mg]/100g bw	
						Uterus	Vagina
Control	Placebo	6	4.3±1.3	3.4±0.6	5	35.3±5.3	23.6±3.8
E_2	0.1µg	6	30.0±3.5*	12.0±2.6*	7	137.0±25.4*	43.4±5.3*
DMT	3.0mg/ kg	6	4.7±1.0	4.6±1.0	7	34.7±9.0	20.0±2.7
DMT	10.0mg/kg	6	6.3±3.0	4.9±1.4	7	37.4±6.0	24.4±6.5
DMT	30.0mg/kg	6	7.1±1.2	6.0±1.0	7	39.0±9.0	23.6±5.0

Tab. 3: Antiestrogenic activity in rats. *Significant compared to estradiol ($p<0.05$, Kruskal-Wallis-Test)

Group	Dose (s.c.)	Rats [n]	Organ weight in [mg]/100g bw	
			Uterus	Vagina
Control E_2	Placebo	6	43.7±7.5*	22.7±5.7*
Estradiol	0.1µg	6	161.5±41.8	50.3±7.5
E_2+DMT	3.0mg/kg	6	130.2±20.0	51.2±7.0
E_2+DMT	10.0mg/kg	6	140.7±30.0	53.4±6.0
E_2+DMT	30.0mg/kg	6	123.4±17.0	48.2±9.0

Tab. 4: Clauberg Assay

Group	Total Dose for 5 days (s.c.) in [mg]	Mc-Phail Index
Placebo	/	1.0
Progesterone P_4	1.0	3.7
DMT	5.0	2.4
P_4+DMT	0.5+1.0	3.5
P_4+DMT	1.5+1.0	3.4
P_4+DMT	5.0+1.0	3.5

THG did neither display (anti-)estrogenic nor (anti-)glucocorticoid activity in our classical preclinical test systems. THG did not inhibit AR activation by testosterone or progesterone receptors activation by progesterone, nor did THG have endogen receptor agonist or antagonist activity [9]. Based on the chemical structure of the compound special emphasize was given to elucidate the progestational and androgen-like activity. Whereas no (anti-)androgenic or (anti-)progestational activity was detected, the compound in fact induced both biologically relevant androgen and progestational activity in all test systems: The progestational activity was analyzed in estradiol primed rabbits after a treatment period of five days ("Clauberg Assay") and in a bioassay to measure the proliferative effects of progesterone on the growth of tubulo-alveolar buds (morphometric evaluation, biochemical DNA, RNA analysis) in the mammary gland in ovariectomized and estrone substituted rats. The androgenic effects of THG were analyzed in the classical orchiectomized mouse and rat model after (s.c.) treatment periods up to 12 days in direct comparison to testosterone propionate (0.1mg/animal/day s.c.): THG induced (a dose-dependent) androgenic effect on prostate and vesicular seminales parameters and LH serum levels in doses of 1.0, 3.0, 10.0mg/animal. This androgenic stimulation could be antagonized by the simultaneous administration of the pure antiandrogen Flutamide (3.0mg) (comparable data to DMT).

4. Discussion and Conclusion

Norbolethone and DMT exert mainly androgen-anabolic effects, whereupon THG was identified to additionally induce progestational modulations. Progestin activity lends itself to negatives such as intensifying certain side effects, including enhanced fat storage, gynecomastia and liver disease (all estrogenic activity associated side effects).

As displayed in Fig. 6, there are similarities between the AR and the progesterone and glucocorticoid receptor. It is assumed that the abused steroids will cause disorders far over the by all means dangerous side effects of androgen anabolic steroids (AAS) individually [10]. Therefore, these compounds may induce severe biomedical side effects and must be considered as perilous drugs, especially when used in high doses for doping purposes.

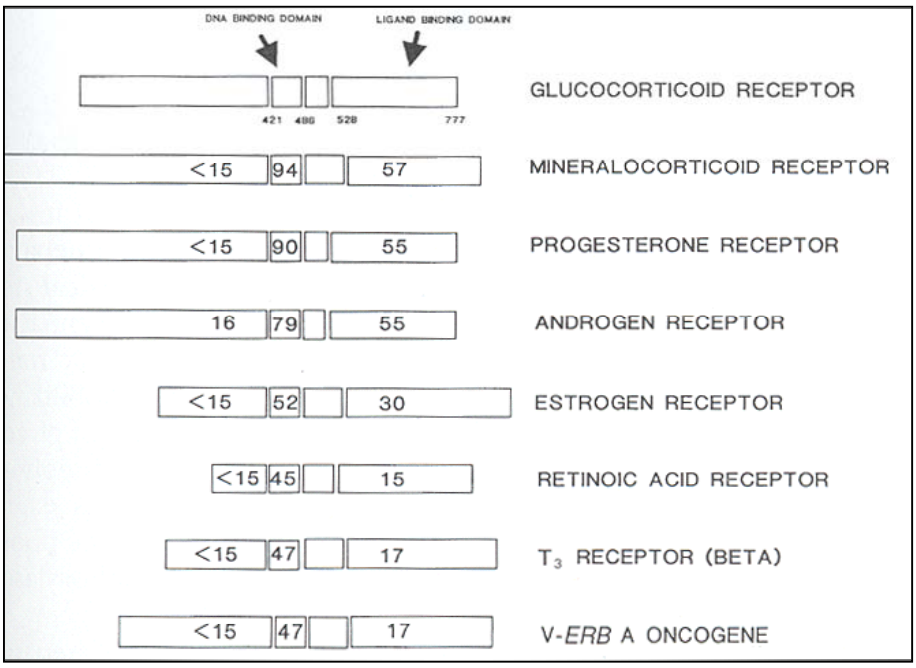

Fig. 6: Domain structures of human hormone receptors and their homology with other steroid receptors (in %).

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Dr. Tsuyuki Nishino - *nishino@sp.tum.de*
Dr. Thorsten Schulz - *schulz@sp.tum.de*
Prof. Dr. Renate Oberhoffer - *oberhoffer@sp.tum.de*
Prof. Dr. Dr. Horst Michna†
Institut für Sport und Gesundheitsförderung
Technische Universität München
Connollystrasse 32
D-80809 München

Prof. Dr. Rudhard K. Müller, em.
Institut für Dopinganalytik und Sportbiochemie
Dresdner Strasse 12
D-01731 Kreischa
E-Mail: *RKMueller.Leipzig@t-online.de*