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New players in the nightwatch research

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Abstract

The metabolism of a variety anabolic steroids frequently misused for doping purposes has been investigated in the last years. This research mainly focused on main and long-term metabolites suitable for detection, but detailed clearance mechanisms have rarely been elucidated. Recent studies on metandienone focused on the identification of 17β-hydroxy-methyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one (NV, **[3]**) as long-term metabolite. It was introduced to the community as "nightwatch" (NV) by Schänzer et al. (Schänzer et al., 2006) in 2006. The metabolic pathway of its generation, however, remained unclear.

In-vitro experiments using Metandienone **[1]** and its Wagner-Meerwein rearrangement product 17,17-dimethyl-18-norandrosta-1,4,13-trien-3-one (NorMD, **[2]**) using different human cytochrome P450 enzymes (CYPs) resulted in hydroxylations of the substrates. Biotechnological synthesis was performed by hydroxylation of NorMD. NV **[3]** was obtained using recombinant strains of the fission yeast *Schizosaccharomyces pombe* expressing the human cytochrome P450 enzyme CYP21. Also CYP3A4 was shown to catalyze this reaction (Zöllner et al., 2010).

Herein, we report the hydroxylation of metandienone itself using the purified human cytochrome P450 enzymes CYP11B1 and CYP11B2. Both enzymes are known to catalyze β-hydroxylations at C-11. Additionally, CYP11B2, also named aldosterone synthase, is known to perform C-18 hydroxylations.

Thus, 18-hydroxymetandienone (17β,18-dihydroxy-17α-methylandrosta-1,4-dien-3-one, 18OH-MD, **[9]**) and 11β-hydroxymetandienone (11β,17β-dihydroxy-17α-methylandrosta-1,4-dien-3-one, 11βOH-MD, **[8]**) were obtained and characterized by GC-MS(/MS). Following Wagner-Meerwein rearrangement, the resulting products could be assigned to NV and 11β-hydroxy-17,17-dimethylandrosta-1,4,13-trien-3-one (11βOH-NorMD, **[6]**).

Following oral administration of either metandienone (25 mg) or NorMD (2 mg) in one human volunteer each the post administration urines were checked for the presence of those hydroxylated metabolites using GC-MS/MS analysis. The GC-MS/MS data of the target analytes are displayed in Table 1. The detection times of the urinary metabolites after metandienone administration are also included in Table 1.

After administration of NorMD **[2]**, NV **[3]**, EpiNV **[4]**, 16βOH-NorMD **[5]**, and 16αOH-NorMD **[7]** were detected in the combined unconjugated and glucuronide (f+g) fractions of the urine samples, while 11βOH-NorMD **[6]** was not detectable. Also not detectable were NorEMD and, rationally, metandienone **[1]**, EMD, 5α- and 5β-THMT as well as the hydroxy-metandienones **[8]** and **[9]**.

Table 1: Retention times (RT), Molecular ions (M+) and ion transitions of the per-TMS derivatives of the steroid target analytes as well as their detection times after single oral administration of metandienone (25 mg, p.o.) and NorMD (2 mg, p.o.)

Analyte	RT [min]	M^+	Ion Transition used for quantitation	Detection time after 25 mg MD
Metandienone **[1]**	14.73	444	206 → 191	-
NorMD **[2]**	7.72	354	339 → 133	-
NV **[3]**	12.91	442	236 → 133	>28 d
EpiNV **[4]**	12.48	442	236 → 133	>28 d
16βOH-NorMD **[5]**	12.71	442	442 → 337	5 d
11βOH-NorMD **[6]**	10.97	442	442 → 337	2.25d
16αOH-NorMD **[7]**	10.75	442	442 → 337	traces
11βOH-MD **[8]**	17.33	532	532 → 206	1.5 d
18OH-MD **[9]**	17.43	532	532 → 206	6 d
EMD	9.26	448	358 → 301	10 d
NorEMD	5.92	358	253 → 185	10 d
5α-THMT	12.18	450	270 → 213	-
5β-THMT	12.40	450	270 → 213	6 d
Epi-MD	12.84	444	206 → 191	4 d
Methyltestosterone (IStD)	15.04	446	301 → 169	(IStD)

The contribution of CYP11B1 and CYP11B2 in human metabolism of metandienone was confirmed by analysis of post-administration samples of metandienone and NorMD. Combined with the results from the previous study (Zöllner et al., 2010), enzymatic pathways were identified that involve CYP21 and CYP3A4 in the hydroxylation of NorMD, while CYP21, CYP3A4 and CYP11B2 take part in NV generation from MD.

The current study represents a valuable contribution to the elucidation of clearance mechanisms of anabolic steroids and also indicates that mainly non-liver CYPs seem to be involved in these processes.

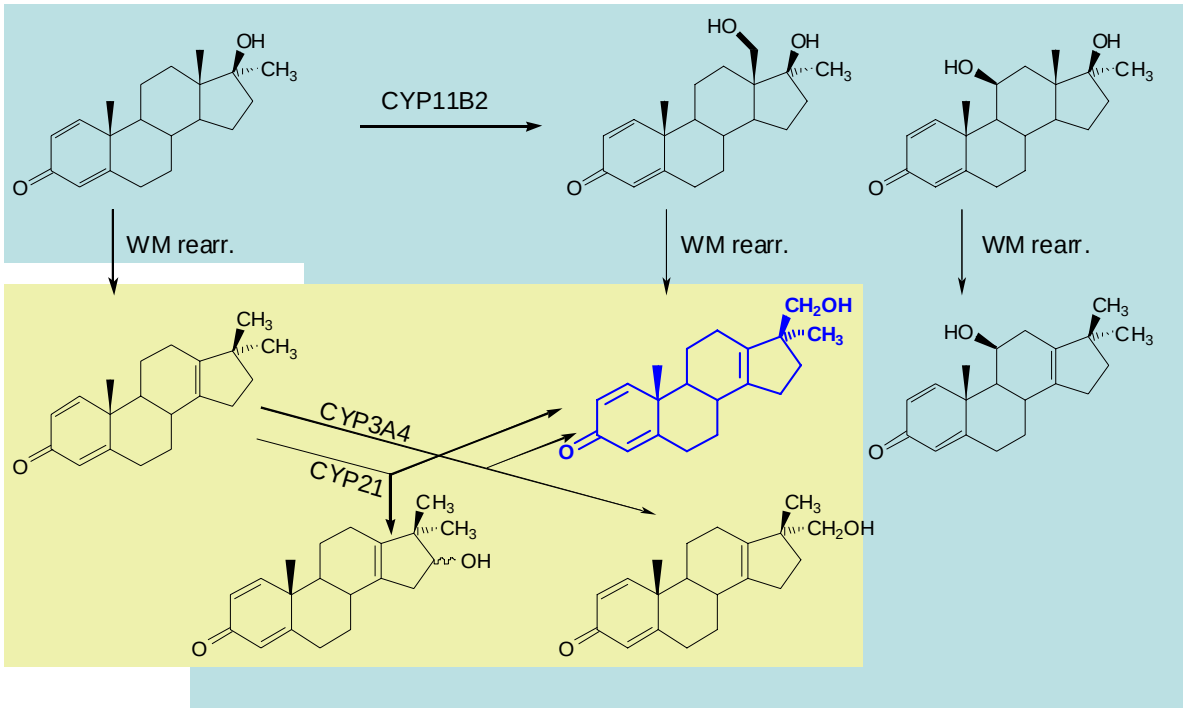

Figure 1: Proposed metabolic pathways of metandienone ***[1]*** *and NorMD* ***[2]*** *leading to 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one (nightwatch, NV* ***[3]****)*

References

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Remark

The detailed results summarizing these investigations will be published elsewhere.