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Disposition of 17β-trenbolone in humans

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ABSTRACT

The urinary excretion and metabolic pattern of 17β-trenbolone, a synthetic anabolic steroid hormone used as a growth promotor for beef cattle in several countries, has been studied in a human subject. For the separation of the metabolites of 17β-trenbolone, a reversed-phase high-performance liquid chromatographic method was established. The method was tested with metabolites obtained from incubation of 17β-trenbolone with rat liver microsomes. Fifteen metabolites could be well separated in one run by using a concave acetonitrile–water–methanol gradient. After ingestion of the tracer-labelled hormone at a dose of 0.04 mg/kg body weight 54% of the administered radioactivity was found in the urine after 26 h and 63% after 72 h. Of the urinary material 54% was present as glucuronides, which contained mostly 17α-trenbolone, 17β-trenbolone and trendione. At least five other polar metabolites, presumably hydroxylated products, were found in smaller amounts, mostly in the unconjugated and sulphated fractions. Thus, the disposition of 17β-trenbolone in humans differs significantly from that in rats, which may have a bearing on the toxicological evaluation of the hormone.

INTRODUCTION

Trenbolone (17β-hydroxy-4,9,11-trien-3-one, β-TBOH, Fig. 1) is a synthetic androgenic steroid hormone. Its acetate (17β-acetoxy-4,9,11-trien-3-one, TBA, Fig. 1) is currently used as a growth promotor in several countries.

The potential exposure of a large population to residues of β-TBOH has raised interest in the toxicological effects, in particular the genetic toxicity, of this compound. Short-term *in vitro* studies on the genotoxicity of β-TBOH were mostly negative [1–3]. However, β-TBOH was reported to transform Syrian hamster embryo (SHE) fibroblasts in culture [4] and to induce micronuclei in these cells [5]. A possible explanation for the effects of β-TBOH in SHE cells may be the formation of genotoxic metabolites.

Previous studies on the biotransformation of TBA in rats and cows [6] have demonstrated a pronounced biliary excretion in both species but a significant difference in the pattern of oxidative metabolites. Whereas 16α-hydroxylation products of β-TBOH and trendione (TBO) (Fig. 1) were major products in rat bile, 17-epi-TBOH (α-TBOH, Fig. 1) predominated in cow bile. In view of these

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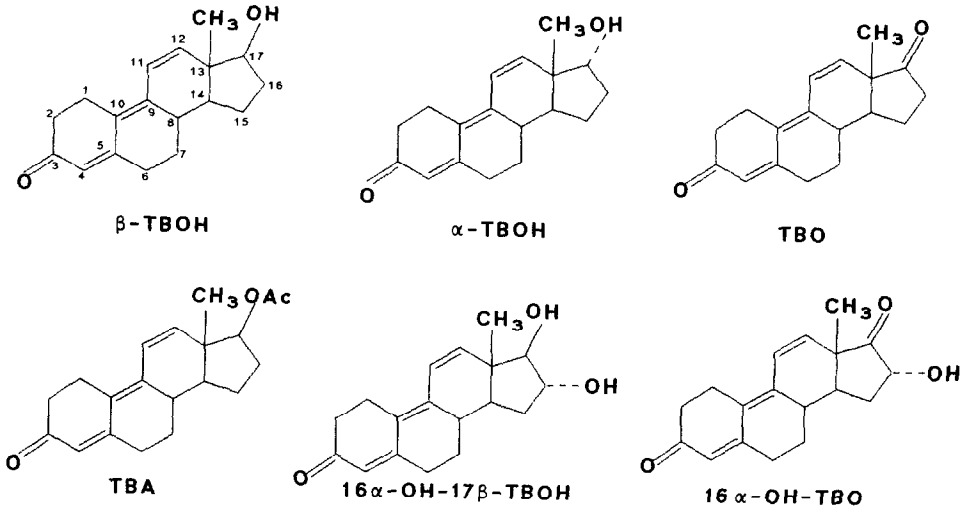

Fig. 1. Structures of β-TBOH, its metabolites, and TBA.

species differences it was of interest to investigate the metabolism of β-TBOH in humans.

EXPERIMENTAL

[6,7-^{3}H]β-TBOH (specific radioactivity 57 Ci/mmol) was kindly provided by Roussel Uclaf (Romainville, France) and purified to >98% by high-performance liquid chromatography (HPLC) (see below) prior to use. Unlabelled β-TBOH, α-TBOH and TBO were also from Roussel Uclaf. All other chemicals were purchased from Boehringer (Mannheim, Germany), Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), Sigma (Munich, Germany) and Promochem (Wesel, Germany) and were of the highest quality available.

For the metabolic study, [^{3}H]-β-TBOH was diluted with β-TBOH to yield a specific radioactivity of 3.6 mCi/mmol. This material, dissolved in 200 μl of ethanol, was injected into a 5-g piece of a fried hamburger and subsequently ingested at a dose of 0.04 mg/kg body weight.

Urine was collected in fractions for 72 h and 0.5-ml aliquots of each fraction were immediately measured in triplicate by liquid scintillation counting (LSC). The remainder was stored at 4°C in a refrigerator.

The fraction of the first 3-h urine contained the highest concentration of radioactivity and was used for the analysis of metabolites. The radioactivity was first adsorbed to an Amberlite XAD-2 column (Serva) and subsequently eluted with methanol. Free and conjugated metabolites were then separated by column chromatography on neutral Al_2O_3 (activity 1, Merck). Unconjugated material was eluted with 96% ethanol, sulphates with water and glucuronides with 40 m*M*

citrate–phosphate buffer (pH 6.0). The radioactivity in each fraction was measured by LSC. Glucuronide and sulphate conjugates were then hydrolysed (24 h, pH 5.2, 37°C) with a mixture of β-glucuronidase and sulphatase (0.5 U *Helix pomatia*, Serva). Deconjugated metabolites were extracted with ethyl acetate, and the organic extract was evaporated under reduced pressure, dissolved in methanol and analysed by reversed-phase HPLC on a Waters gradient apparatus equipped with a Berthold radioactivity flow detector (Model LB 506C) based on LSC. A 250 mm × 4 mm I.D. column packed with Zorbax ODS 5 μm (Bischoff, Leonberg, Germany) was operated at a flow-rate of 1.0 ml/min. Solvent A was water–methanol (9:1, v/v) and solvent B acetonitrile. A concave gradient (Waters Millipore automated gradient controller 680, curve 8) was changed from 15% B to 37% B in 25 min, held at 37% B for 10 min and then changed to 100% B in 5 min. The UV detector was set to 340 nm, and the radioactive peaks were quantitated by the Berthold data system and collected manually for gas chromatographic–mass spectrometric (GC–MS) analysis. In this manner, fifteen ^{3}H-labelled oxidative β-TBOH metabolites obtained from an incubation of [^{3}H]-β-TBOH with rat liver microsomes (B. Spranger, unpublished data) could be completely separated in one HPLC run. Five identical HPLC analyses of the microsomal [^{3}H]-β-TBOH metabolites demonstrated an accuracy of >97%. Only peaks containing >2% of the recovered radioactivity were considered metabolites and clearly discriminated from noise.

Identification of the metabolites was based on cochromatography with authentic reference substances in HPLC and GC–MS. For GC–MS, HPLC fractions were evaporated to dryness and dissolved in N,O-bis(trimethylsilyl)acetamide. After standing for 24 h at room temperature, the trimethylsilyl derivatives of the samples were analysed on a Hewlett Packard 5971A GC–MS system using a HP-1 methylsilicone 0.33 μm fused-silica capillary column (12 m × 0.22 mm I.D.). The injector temperature was 200°C and the detector temperature 290°C. The column temperature was raised from 70°C to 300°C at 10°C/min and kept at the upper temperature for 10 min. Electron-impact mass spectra (70 eV) were taken at 1-s intervals and stored by the HP data system Vectra QS20, MS CHEM Station.

RESULTS

Urinary excretion and conjugation pattern

After a single oral dose (0.04 mg/kg body weight, 3.6 mCi/mmol) of [^{3}H]-β-TBOH, 63% of the administered radioactivity was excreted in the urine after 72 h (Fig. 2). The 24 h urine contained *ca.* 50% of the dose.

More than 90% of the radioactivity could be extracted from the urine by liquid–solid extraction on Amberlite XAD-2. More than 85% of the excreted radioactivity was recovered after separation of free, sulphated and glucuronidated metabolites by column chromatography on neutral Al_2O_3.

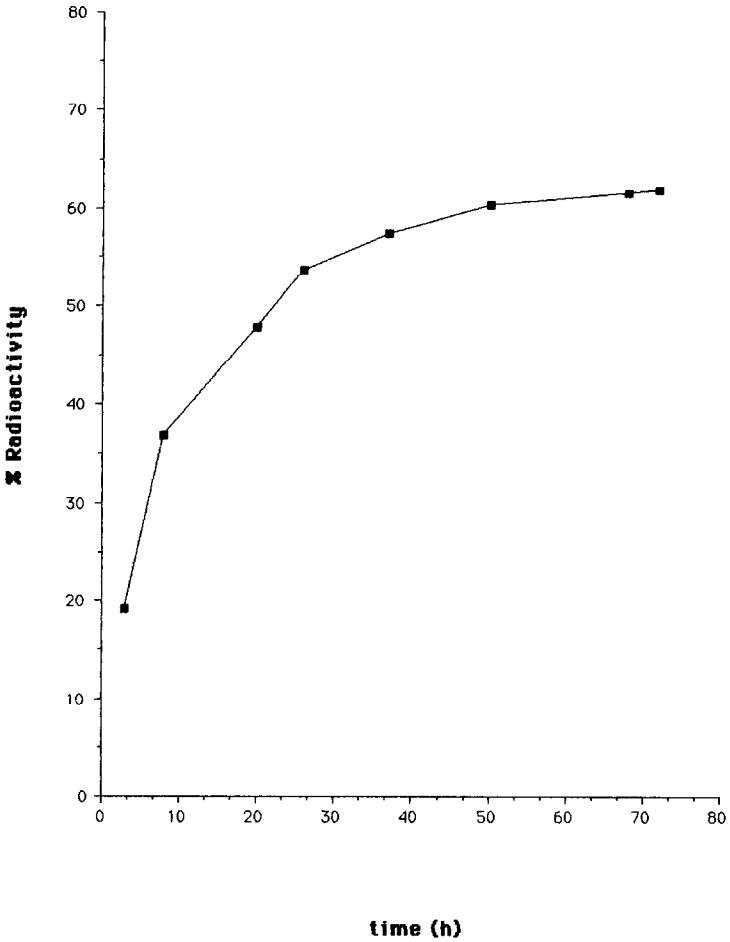

Fig. 2. Urinary excretion of radioactivity after oral administration of [^{3}H]-β-TBOH.

The major fraction of the urinary radioactivity was present as glucuronides (54.7%). Sulphates and unconjugated metabolites represented 20.9 and 24.4%, respectively. After enzymic deconjugation of the glucuronides and sulphates, 55–65 and 78–85%, respectively, of the radioactivity could be extracted into the organic phase, which was then analysed by HPLC for the metabolic pattern.

Oxidative metabolites

According to HPLC analysis, the pattern of oxidative metabolites differed in the three fractions (Fig. 3). Unconjugated material was very complex, consisting of β-TBOH, α-TBOH, TBO and several unknown polar metabolites. Sulphates contained mostly two unknown metabolites, and glucuronides contained some β-TBOH together with a large amount of α-TBOH. The HPLC profile of the

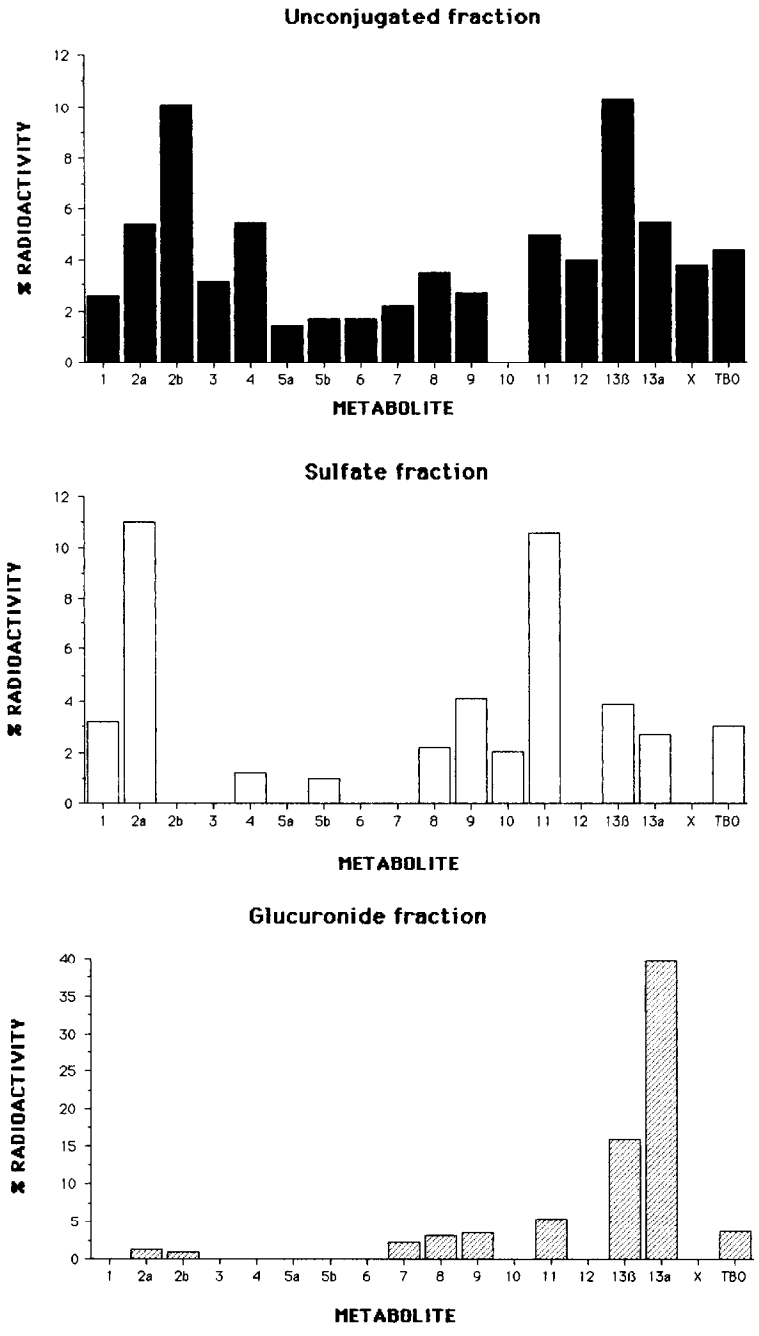

Fig. 3. Metabolic pattern of β-TBOH in the unconjugated, sulphated and glucuronidated fractions.

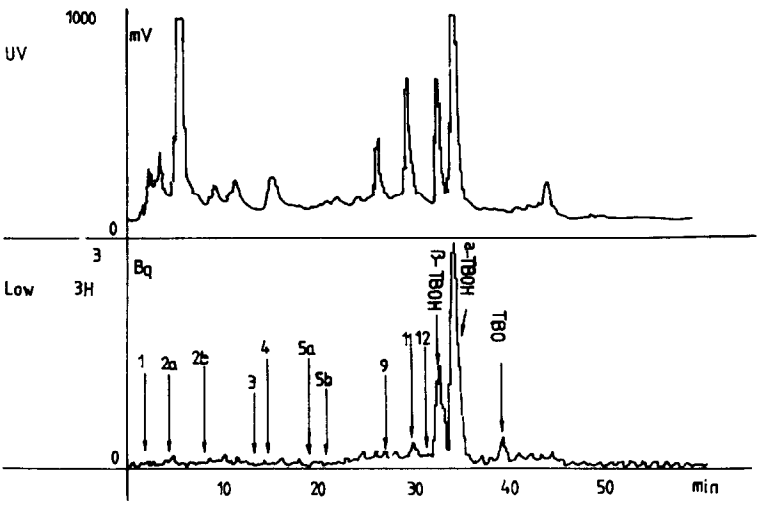

Fig. 4. HPLC profile of the hydrolysed glucuronide fraction. Numbers indicate retention times of β-TBOH metabolites found in the unconjugated fraction.

glucuronide fraction after enzymic hydrolysis is depicted in Fig. 4. In addition to cochromatography with authentic reference compounds in HPLC, the identification of the urinary metabolites β-TBOH, α-TBOH and TBO was carried out by GC–MS (Fig. 5).

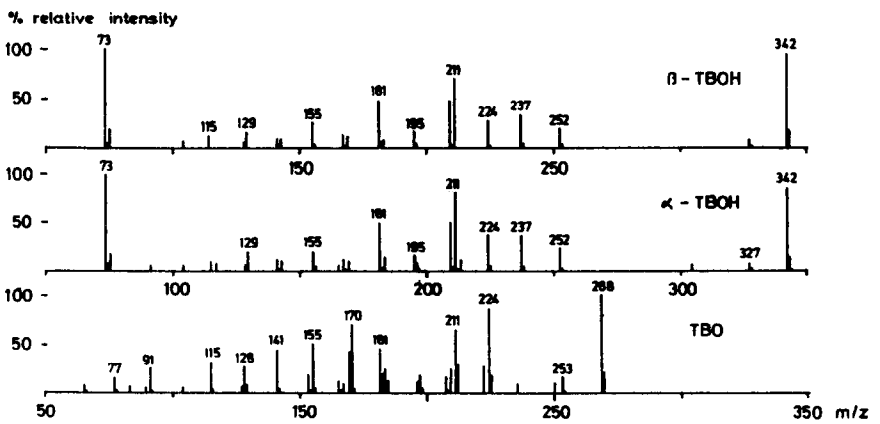

Fig. 5. Electron-impact mass spectra of urinary β-TBOH metabolites. Compounds were analysed as trimethylsilyl derivatives. Molecular masses: TBOH, 342; TBO, 268.

DISCUSSION

The disposition of β-TBOH in humans is of interest because its acetate (TBA) is widely used as growth promotor, particularly in ruminant species [7]. After administration, TBA is rapidly hydrolysed to its active form, β-TBOH [8]. Therefore β-TBOH must be considered to be the major residue of TBA in meat.

In our study, the recovery of more than 60% of an oral dose of [^{3}H]-β-TBOH after a 70-h collection period demonstrates that urine is the major route of excretion for β-TBOH and its metabolites in humans. This is in contrast to the biliary excretion that predominates in rats and cows [6].

In all three species, the major fraction of the metabolites was excreted as glucuronides. Whereas in rats and cows the unconjugated fraction accounted only for a minor part, in human urine free metabolites and sulphates were found in equal amounts.

A reversed-phase HPLC system has been developed, which allows the separation of fifteen β-TBOH metabolites in a single run. By using this method and combined GC–MS, it was found that the pattern of oxidative β-TBOH metabolites in human urine differs significantly from that in rats. Whereas rat bile contained mostly 16α-hydroxy-17β-TBOH and 16α-hydroxy-TBO, the major metabolite in human urine is 17α-TBOH (epi-TBOH).

In this respect the biotransformation of β-TBOH in humans resembles that reported for cows [6]. The metabolic conversion of β-TBOH into its α-epimer is known to decrease the androgenic and anabolic potency significantly [9]. This means that metabolism of β-TBOH in humans should lead to inactivation. However, the toxicological significance of the polar β-TBOH metabolites found in small amounts in humans remains to be elucidated. In particular, it should prove interesting to see whether any of these metabolites are related to the genotoxic potential of β-TBOH in SHE cells. The observation of β-TBOH residues covalently bound to proteins in cattle tissue [10] indicates that reactive intermediates are indeed formed in β-TBOH metabolism.

ACKNOWLEDGEMENTS

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