"Metabolism of anabolic steroids by adrenocortical cytochromes P450– search for new long-term doping markers"

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Project overview:

Because of their performance-enhancing effects anabolic androgenic steroids (AAS) have been prohibited by the International Olympic Committee since 1976. However, they still belong to the most frequently detected substances in doping tests and improvement of the detection capabilities is one major aim in anti-doping research. This includes a better understanding of their metabolism in the human body and the discovery of long-term metabolites. In this context cytochromes P450s (P450s), who contribute to the biotransformation and subsequent renal clearance of exogenous compounds as well as to the biosynthesis of endogenous steroids, are a promising target for the investigation of the metabolic pathways of exogenous steroids like AAS. While the metabolism of xenobiotics by liver P450s is well studied, their biotransformation by adrenal P450s is nearly unknown. Consequently, our aim is to study the metabolism of AAS by P450s from the human adrenal cortex, in order to provide new reference material for doping analysis. Adrenal P450s are involved in the biosynthesis of steroid hormones and only very few cases regarding the metabolism of xenobiotics by these P450s have been described so far. Therefore, our project aims to investigate the in vitro metabolism of AAS by these P450s employing the world-wide unique set of purified proteins available in our lab.

The applicability of discovered novel metabolites as (long-term) markers in doping tests will be examined by comparison with the urinary excretion profile in a mass spectrometry analysis. For metabolites, that turn out to be useful biomarkers, a larger scale production by genetically modified bacterial cell factories will be developed to create a basis for the supply of these metabolites to other laboratories for incorporation in routine doping analysis.

Results and Conclusions:

Anabolic androgenic steroids (AAS) are frequently misused for doping purposes due to their performance enhancing effects. In the human body, they are metabolized, as all other drugs, in the liver to facilitate their clearance via the urine. The detection of AAS metabolites in the urine of an athlete by doping controls can demonstrate a doping delict. Most metabolic reactions in the liver are carried out by a group of proteins, or enzymes, called cytochromes P450. Closely related enzymes, which also belong to the cytochrome P450 group, are responsible for the synthesis of natural steroid hormones in the adrenal, in reproductive and some other tissues. The

question arises whether these steroid-synthesizing enzymes can also contribute to the metabolism of AAS and produce alternative target metabolites for doping controls. The six human steroid-synthesizing enzymes (CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP19A1 and CYP21A2) were produced in genetically modified bacteria (Escherichia coli) and purified to evaluate their capability to metabolize the AAS oral-turinabol (OT), stanozolol and oxandrolone. Three of the enzymes (CYP11A1, CYP11B1 and CYP11B2) were found to metabolize OT and the structures of the emerging metabolites were elucidated by different methods (nuclear magnetic resonance spectroscopy, liquid chromatography tandem mass spectrometry). We identified five new OT metabolites (11β- hydroxy-OT, 11β,18-dihydroxy-OT, 11β-hydroxy-OT-18-aldehyde, 2-hydroxy-OT and 2,16-dihydroxy-OT) that have not been described in the literature to date and therefore represented interesting candidates as target analytes for doping controls. However, when we analyzed urine from an OT administration study, none of the compounds could be detected. This suggests that the new OT metabolites are further metabolized by additional enzymes prior to their excretion with the urine, which hampered their detection. In conclusion, the new OT metabolites cannot be used themselves to demonstrate a doping delict, but can serve as intermediates for the synthesis of their downstream metabolites, that might be excreted with the urine.