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In vitro formation of fenoterol sulfates using different tissue preparations and recombinant sulfotransferases

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Abstract

Fenoterol is a β_2-agonist and listed on “The 2012 Prohibited List” of the World Anti-Doping Agency (WADA) in the category S3 “Beta-2 Agonists”. Fenoterol is a direct-acting sympathomimetic agent with β_2-adrenoreceptor stimulant activity. Following oral administration, the major metabolic pathways are sulfation and glucuronidation. Sulfotransferases 1A (SULTs1A) are known to catalyse the reaction of phenols (amongst others) with 3'-phosphoadenosine- 5'-phosphosulfate (PAPS). SULTs are distributed in different tissues, and are locatable in the cytosol of liver, lung, intestine and kidney.
In the present study, we report on the *in vitro* sulfation of fenoterol in human S9 fractions of different tissues. Additionally, sulfation was performed with the recombinant enzymes SULT1A1 and SULT1A3.
The *in vitro* approach was optimised for each tissue or recombinant enzyme concerning incubation time, PAPS concentration and substrate concentration, respectively. The analytes fenoterol, mono-sulfoconjugated fenoterol and bis-sulfoconjugated fenoterol were measured without sample clean-up by LC-MS/MS after protein precipitation.
Due to the three phenolic groups, different sulfation products are possible. In total, three different sulfoconjugates were detected, sulfoconjugate (1) and (2) were observed in each assay, whereas sulfoconjugate (3) was generated in substrate-to-PAPS-ratio dependent manner (structures see figure 1).
Additionally, excretion study urine specimens were examined for sulfoconjugates. All of the *in vitro* synthesised conjugates were detected in these samples. In order to potentially discriminate the route of administration, excretion studies with inhaled and oral administered fenoterol were performed and examined for the formation of the sulfoconjugates.

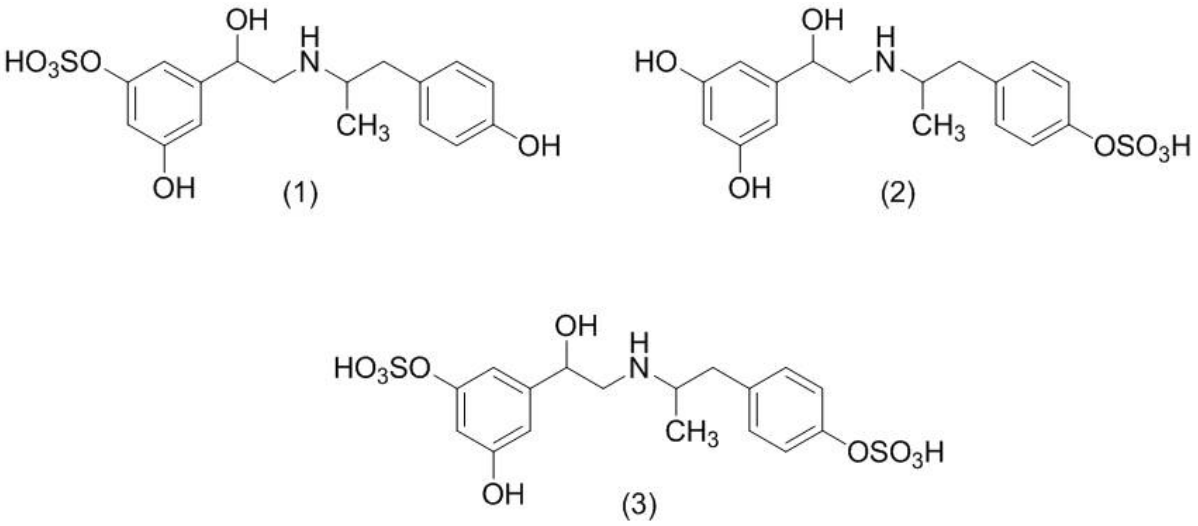

Figure 1: Structures of in vitro synthesized fenoterol sulfoconjugates

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