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Estrone sulfate and dehydroepiandrosterone sulfate: Transactivation of the estrogen and androgen receptor

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ABSTRACT

Dehydroepiandrosterone sulfate (DHEAS) and estrone sulfate (E1S) are two of the most abundant steroids in the human circulation. The enzyme steroid sulfatase (STS) cleaves the sulfate group of DHEAS and E1S leading to biosynthesis of endogenous hormones such as testosterone and estrone.

In the current study we aimed at determining the effect of E1S and DHEAS on estrogen receptor (ER) and androgen receptor (AR) transactivation. Using luciferase reporter gene assays, the ER and AR transactivities of E1S and DHEAS were determined by direct cell exposure; as well as upon extraction from human serum using a method to extract perfluorinated alkyl acids (PFAAs).

By direct cell exposure, both E1S and DHEAS transactivated the ER and the AR in dose-dependent manners. The DHEAS-induced AR transactivity could be abolished by the STS inhibitor STX64. Immunoassay analysis confirmed the presence of E1S and DHEAS in the serum PFAA extracts with mean recoveries below 2.5%. For the PFAA extracts of human male and female serum, only the AR was significantly transactivated. The AR transactivity of the sulfated steroids in the extracts was abolished by STX64 to obtain the net PFAA induced xenohormone transactivity, but further cleanup might be needed at high concentrations of E1S.

1. Introduction

Dehydroepiandrosterone sulfate (DHEAS) is the most abundant steroid in the human circulation [1], and it is an important source for estrone (E1) and estradiol (E2) biosynthesis during pregnancy [2]. In 1984, Orentreich et al. reported a normal serum level of DHEAS ranging from approximately 200 ng/mL in old women to approximately 5500 ng/mL in young men [1]. Estrone sulfate (E1S) is another highly abundant steroid in human serum with concentrations that are ten to twenty fold higher than E1 and E2, being in the range of 0.13 ng/mL in serum from postmenopausal women to 105 ng/mL in pregnant women (third trimester) [3].

Perfluorinated alkyl acids (PFAAs) are a group of very persistent bio-accumulative, man-made compounds. Recently, we developed a method to extract the PFAAs from human serum with simultaneous removal of uncharged endogenous sex hormones such as E1, E2, estriol, estetrol and testosterone [4]. Compounds other than the PFAAs might be present in the serum extracts, but if so, these compounds are expected to have similar physicochemical properties as the PFAAs such as being amphiphilic and contain an anionic functional group. Based on the chemical structure of DHEAS and E1S (Table 1), we hypothesize the presence of these metabolites in the PFAA serum extracts.

The metabolism of DHEAS and E1S to their unconjugated counterparts – dehydroepiandrosterone (DHEA) and E1, respectively – is mediated by the enzyme Steroid Sulfatase (STS). DHEA and E1 can then be further transformed to more potent estrogens such as androstenediol and E2, respectively [5–7]. Several compounds have been tested for the inhibition of STS, and one of the most potent STS inhibitors *STX64* (also known as Irosustat and 667 COUMATE) has entered human trials for treatment of advanced hormone-dependent breast cancer in postmenopausal women [6,7].

Abbreviations: AR, androgen receptor; DC, dextran/charcoal; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; E1, estrone; E1S, estrone sulfate; E2, 17β-estradiol; ER, estrogen receptor; FCS, fetal calf serum; HF, hydroxyflutamide; LDH, lactate dehydrogenase; LOEC, lowest observed effect concentration; MOEC, maximum observed effect concentration; PFAA, perfluorinated alkyl acid; STS, steroid sulfatase.

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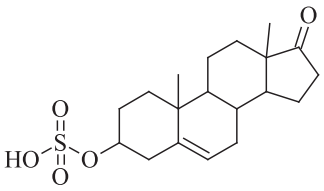
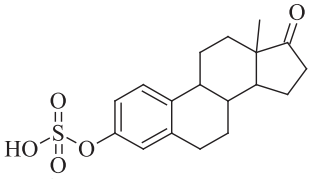
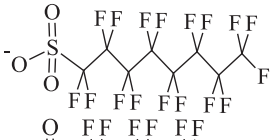
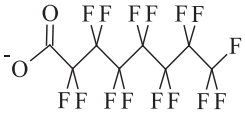
Table 1
Chemical structures of dehydroepiandrosterone sulfate, estrone sulfate and two perfluorinated alkyl acids (PFOS and PFOA).

	Compound	Structure
DHEAS	Dehydroepiandrosterone sulfate	
E1S	Estrone-3-sulfate	
PFOS	Perflurooctane sulfonate	
PFOA	Perfluorooctanoate	

In vivo studies in ovariectomized rats have shown that STX64 is not estrogenic itself [5].

In the present study we aimed to (*i*) determine whether the ER and the AR are transactivated *in vitro* by direct cell exposure to DHEAS and E1S, (*ii*) determine whether DHEAS and E1S are present in human serum extracts using our recently developed PFAA extraction method [4], (*iii*) determine whether the responses of DHEAS and E1S alone and in the serum extracts can be inhibited by the STS inhibitor STX64, and (*iv*) to evaluate whether the transactivities induced by the PFAAs in the serum extracts can be analyzed without interference from DHEAS and E1S.

2. Experimental

2.1. Reagents

Solid standards of sodium dehydroepiandrosterone-3-sulfate, potassium estrone-3-sulfate, STX64, and ICI 182,780 were purchased from Sigma–Aldrich (Brøndby, Denmark). Hydroxyflutamide (HF) was purchased from MicroMol GmbH (Luckenwalde, Germany). 50 mM working stocks were prepared by dissolving DHEAS, E1S and STX64 in DMSO (VWR – Bie & Berntsen, Denmark) and ICI and HF in 96% ethanol (VWR – Bie & Berntsen, Denmark). The working stocks were stored at 5 °C.

2.2. Serum samples

Pooled serum from young healthy Danish men and young healthy Danish women, respectively, was kindly donated by the blood bank at Skejby Hospital (Aarhus, Denmark). The pooled serum of each sex was divided into 3 mL aliquots in Cryo tubes (Nunc, Roskilde, Denmark) and stored at −80 °C.

2.3. Methods

2.3.1. Serum PFAA extraction method

The method used for extraction of PFAAs from human serum consists of solid phase extraction, liquid/liquid extraction, HPLC fractionation and weak anion exchange. The method was validated by extraction of seven persistent PFAAs from spiked human male serum obtaining mean recoveries between 49.6% and 78.6%. Upon extraction, the PFAA serum extracts were free of most endogenous sex hormones such as E1, E2, estriol, estetrol and testosterone. The details have been described previously [4]. PFAAs are present in many materials including laboratory equipment, and it is therefore difficult to completely avoid contamination during extraction [8]. For all extractions, double distilled water (distilled on clean glassware) was extracted in parallel with the serum samples. This extraction blank is used as a control for contamination of the samples during the extraction.

2.3.1.1. Recovery analysis. Two female serum samples were spiked with DHEAS to final concentrations of 3.68 and 0.92 μg/mL, respectively; and two male serum samples were spiked with E1S to the final concentrations 100 and 10 ng/mL, respectively. Female serum was chosen for DHEAS recovery analysis and male serum for E1S recovery analysis to get as low background levels as possible. These four samples and two unspiked serum samples (one male and one female) were then extracted using the serum PFAA extraction method [4]. The final extract containing the actual PFAA mixture was collected, evaporated to near dryness, and stored at −20 °C until immunoassay analysis.

2.3.2. Immunoassays

The final serum PFAA extracts were dissolved in 80 μL double distilled water. The DHEAS and E1S concentrations were measured twice for each serum sample and in triplicates for the dissolved serum PFAA extracts using immunoassays according to the manufacturers' instructions. The E1S ELISA kit was purchased from Biotech IgG (Copenhagen, Denmark), and the DHEAS ELISA kit was developed by Biovendor (Brno, Czech Republic).

2.3.3. ER and AR transactivity assays

The stable transfected MVLN cell line (kindly provided by M. Pons, France), carrying a reporter vector including the estrogen response element in front of the luciferase gene [9,10], was used for determination of the ER-transactivity as described earlier [4,11–13]. MVLN cells were seeded in white 96-well microtiter plates (Perkin Elmer) with a density of approximately 8.5×10^4 cells per well and cultured in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM, Lonza, Bionordika) media containing 1% dextran/charcoal (DC) treated fetal calf serum (FCS). The next day, the compounds or serum extracts were dissolved in DMEM with 0.5% DC-FCS and added to the wells in triplicate. After incubation for another 24 h, the DMEM was removed and the cells were lysed in 50 μL lysis buffer and the luciferase activity was measured on a BMG LUMIstar Omega microplate reader (RAMCON, Denmark). The protein content in the lysed cells was determined upon addition of 50 μL fluorescamine diluted in acetonitrile (500 mg/L) followed by fluorometric measurements in the Wallac VICTOR2 (Perkin Elmer, USA) at 355/460 nm wavelength. The measured luciferase data were then corrected for cell density using the protein measurements.

The AR transactivity was determined in the Chinese Hamster Ovary cells (CHO-K1) by transient co-transfection with the mouse mammary tumour virus–luciferase (MMTV–LUC) reporter vector carrying the MMTV response element in front of the reporter luciferase gene (kindly provided by Dr. Ronald M. Evans, Howard Hughes Medical Institute, San Diego, CA, USA) and the AR expression plasmid pSVAR0 (kindly provided by Dr. A.O. Brinkmann, Erasmus University, Rotterdam, The Netherlands). The assay was performed as previously described [14,15]. In short; 24 h before transfection, CHO-K1 cells were seeded in white 96-well microtiter plates (Perkin Elmer) with a density of approximately 8000 cells