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The six most widely used selective serotonin reuptake inhibitors decrease androgens and increase estrogens in the H295R cell line

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) used as first line of treatment in major depressive disorder (MDD) are known to exert negative effects on the endocrine system and fertility. The aim of the present study was to investigate the possible endocrine disrupting effect of six SSRIs, fluoxetine, paroxetine, citalopram and its active enantiomer escitalopram, sertraline and fluvoxamine using the OECD standardized and validated human *in vitro* adrenocortical H295R cell assay. All the major steroids, including progestagens, corticosteroids, androgens and estrogens were analysed using a fully validated LC-MS/MS method. All 6 SSRIs were found to exert endocrine disrupting effects on steroid hormone synthesis at concentrations just around C_{max}. Although the mechanisms of disruption were all different, they all resulted in decreased testosterone levels, some due to effects on CYP17, some earlier in the pathway. Furthermore, all SSRIs relatively increased the estrogen/androgen ratio, indicating stimulating effects on the aromatase. Our study demonstrates the potential of SSRIs to interfere with steroid production in the H295R cells around C_{max} levels and indicates that these drugs should be investigated further to determine any hazards for the users.

1. Introduction

Several pharmaceuticals have been developed to attenuate the symptoms of depression. Selective serotonin reuptake inhibitors (SSRIs) are the first line treatment of major depressive disorder (MDD) and they are far more prevalent than other antidepressants such as tricyclic antidepressants (TCA) and selective noradrenalin reuptake inhibitors (SNRIs) (Nierenberg et al., 2007; Werner and Covenas, 2010). MDD is a mental disorder characterized by a persistent low mood and low self-esteem. MDD may also influence sleeping and eating habits (Nestler et al., 2002). SSRIs are speculated to exert their effect by blocking the serotonin reuptake transporter, hence decreasing the reuptake of serotonin to the presynaptic neuron by which the concentration of serotonin in the synaptic cleft is increased. Elevated serotonin levels in the cleft are assumed to attenuate depression (Lindqvist et al., 2015).

Fluoxetine was the first SSRI sold and later other SSRIs have been introduced. Initially, the SSRIs were considered free of adverse effects, but after marketing some side effects have been uncovered. Some of these effects are nausea, dry mouth, appetite changes and sexual dysfunction (Ferguson, 2001). The most sold SSRIs are fluoxetine, sertraline, paroxetine, fluvoxamine and citalopram. Citalopram is a racemic mixture of R- and S-citalopram, but only S-citalopram is therapeutically active. Consequently, the S-enantiomer is also marketed, as escitalopram. The Institute for Rational Pharmacotherapy (IRF) recommends citalopram/escitalopram and sertraline as first choice for treating moderate and severe depressions, because their effect and adverse profile are assumed to be better than fluoxetine and paroxetine (IRF, 2010).

Altered sex hormone production in humans may be caused by a combination of lifestyle factors, anatomical conditions, autoimmune conditions and environmental factors, including endocrine disruptors (Hauser et al., 2015; Skakkebaek et al., 2001). In recent years, the focus on drugs as endocrine disruptors has increased (Winther et al., 2013; Guldvang et al., 2015; Holm et al., 2015, 2016; Kristensen et al., 2016; Sørensen et al., 2016) and studies indicate that adverse effects of SSRIs may be associated with the endocrine system since sexual disorders are common in SSRI users. Between 30 and 60% of all SSRI users have been reported to suffer from sexual dysfunction (Gregorian et al., 2002). In men, this includes low levels of testosterone (TS), luteinizing hormone (LH), and follicle stimulating hormone (FSH), and elevated level of prolactin (Safarinejad, 2008). In women, SSRI use has been associated with breast enlargement and changes in the length of the menstrual cycle (Amsterdam et al., 1997; Steiner et al., 1997). It has also been suggested that binding of serotonin to the brain serotonin (5-HT_2) receptor directly cause decreased libido (Ferguson, 2001). Another explanation may be that increased peripheral serotonin inhibits nitric oxide (NO) levels, which normally relaxes the smooth muscles of the

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vasculature. Inhibition of NO may thus cause decreased blood supply to the sexual organs (Kennedy and Bagby, 2000).

Few studies have investigated the relation between SSRIs and steroid production. A previous study observed a decrease in TS in the H295R cell line during exposure to sertraline, fluoxetine, paroxetine, citalopram and fluvoxamine (Jacobsen et al., 2015) but the effects of these SSRIs on the complete steroidogenesis is not known. In the present study we investigated six SSRIs (paroxetine, fluoxetine, citalopram, escitalopram, sertraline and fluvoxamine) in the H295R cell line to clarify how these drugs alter the steroid production. This cell line is capable of *de novo* synthesis of all the major steroids in the entire steroid pathway including progestagens, corticosteroids, androgens and estrogens. Consequently, analyzing the complete steroid profile in this cell line during SSRI exposure will provide a detailed overview of the effects of these drugs on the native steroidogenic pathway. In total, we analysed 16 steroids (4 progestagens, 6 corticosteroids, 4 androgens and 2 estrogens), covering all major steroids in the human steroidogenesis. An overview of the mammalian steroidogenesis and the involved enzymes is shown in Fig. 1. The chemical structures and selected physicochemical properties of sertraline, citalopram/escitalopram, fluoxetine, paroxetine and fluvoxamine are shown in Table 1.

2. Materials and methods

2.1. H295R steroidogenesis assay

The H295R steroidogenesis assay was conducted in accordance with the OECD (2011) guideline and Nielsen et al. (2012) with minor modifications. The cells were grown at 37 °C with 5% CO_2 in 75 cm^3 flasks with DMEM/F12 growth media with 1% ITS + premix and 2.5% Nu-serum. The media was changed every second day and the culture was trypsinated and sub-cultured when it reached a confluency of 75–90%. The cells used for experimentation were in passage 4–12 (OECD, 2011).

The culture was trypsinized for plating when an acceptable confluency of approximately 90% was reached. The cells were then counted on a bürker-türk cell counter and diluted with growth media to a concentration of $3 \cdot 10^5$ cells/mL. 1 mL cell culture was seeded in each of the 24 wells. The cells were allowed to attach to the wells for 24 h incubation at 37 °C under 5% CO_2. A serial dilution of the six SSRIs with dimethyl sulfoxide (DMSO), which is the preferred solvent in the H295R assay (OECD, 2011) was prepared to achieve different experimental concentrations: Fluoxetine: 0.001, 0.002, 0.03, 0.1, 0.314, 1, 3.75, 7.5, 10 and 20 μM; paroxetine: 0.03, 0.1, 0.2, 0.3, 1, 2, 3, 10 and 15, 20 μM; citalopram: 0.03, 0.1, 0.3, 1, 3.1, 10, 31.4 and 50 μM; escitalopram: 0.0002, 0.002, 0.02, 0.2, 2, 20, 50 μM; sertraline: 0.01, 0.03, 0.1, 0.3, 1.0, 3.1 and 7.5 μM and fluvoxamine: 0.1, 0.3, 1, 3, 10, 31 and 43. The dilutions were prepared so that the final DMSO concentration in the wells was maximum 0.1%, which is recommended in the guideline (OECD, 2011).

For each plate, 7 concentrations were added to the wells in triplicates. Furthermore, 0.1% DMSO (n = 3) was added as a solvent control (SC) to measure the background steroid hormone production. Samples were then incubated. After 48 h, 950 μL cell medium was collected from each sample and 5 ng (50 μL of a 0.1 μg/mL) internal standard (IS) was added. The IS contained the following deuterated standards: d_7-androstenedione (ANd_7), d_4-estrone ($E1d_4$), d_5-17β-estradiol (β-$E2d_5$), d_8-corticosterone ($COSd_8$), d_8-11-deoxycorticosterone (11-deoxy$COSd_8$), d_9-progesterone ($PROGd_9$), d_3-testosterone (TSd_3), d_3-

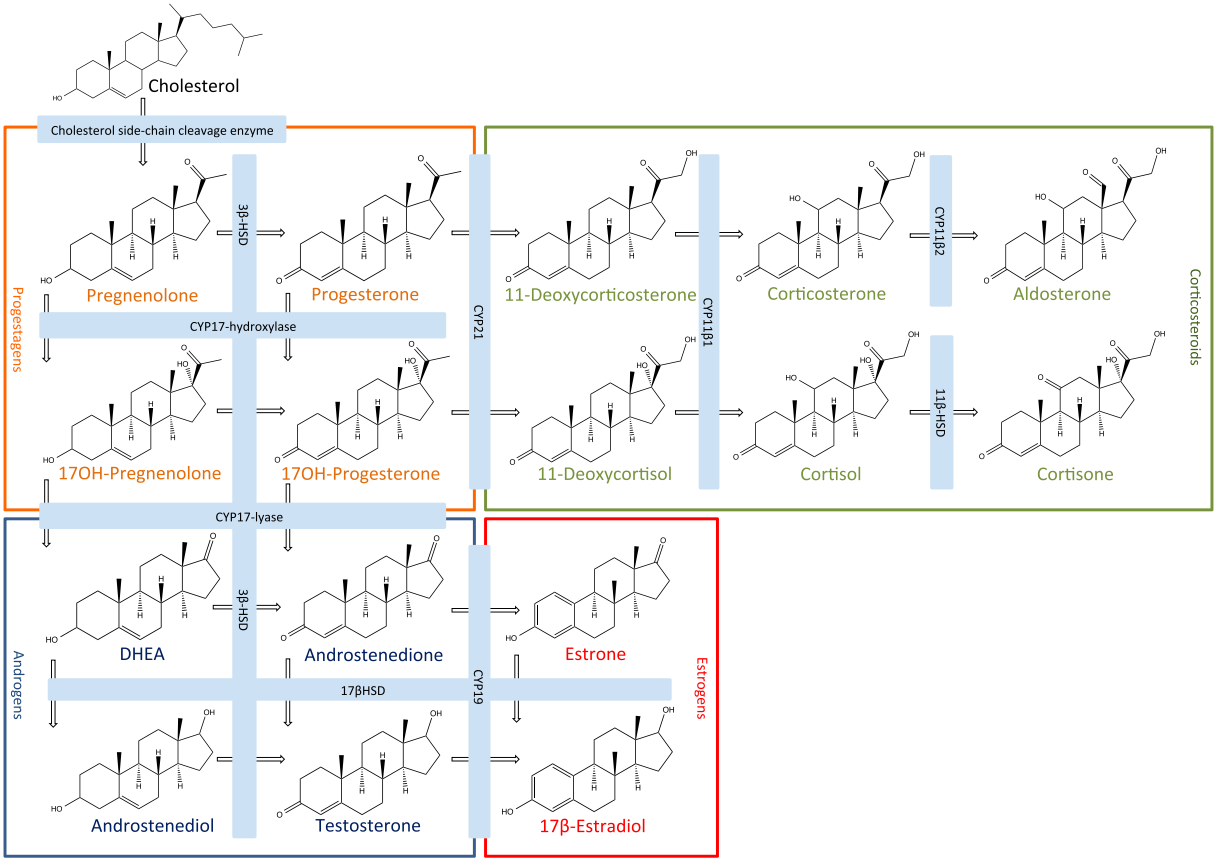

Fig. 1. Overview of the mammalian steroidogenesis showing the chemical structures of all major steroids. CYP: Cytochrome P-450 enzyme. HSD: Hydroxysteroid dehydrogenase. Blue boxes indicate the enzymes involved in the pathway.