The Anabolic Androgenic Steroid Fluoxymesterone Inhibits 11β-Hydroxysteroid Dehydrogenase 2–Dependent Glucocorticoid Inactivation

Cornelia Fürestenberger,*† Anna Vuorinen,‡§ Thierry Da Cunha,*† Denise V. Kratschmar,*† Martial Saugy,¶|| Daniela Schuster,‡§ and Alex Odermatt*†‡†

*Swiss Center for Applied Human Toxicology and ‡Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland; §Institute of Pharmacy/Pharmaceutical Chemistry and ¶Center for Molecular Biosciences Innsbruck-CMBI, University of Innsbruck, A-6020 Innsbruck, Austria; †Swiss Laboratory for Doping Analyses, University Center of Legal Medicine, Geneva-Lausanne, Switzerland; and ||Centre Hospitalier Universitaire Vaudois, University of Lausanne, 1066 Epalinges, Switzerland

†To whom correspondence should be addressed at Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland. Fax: +41 61 267 1515. E-mail: alex.odermatt@unibas.ch.

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Anabolic androgenic steroids (AAS) are testosterone derivatives used either clinically, in elite sports, or for body shaping with the goal to increase muscle size and strength. Clinically developed compounds and nonclinically tested designer steroids often marketed as food supplements are widely used. Despite the considerable evidence for various adverse effects of AAS use, the underlying molecular mechanisms are insufficiently understood. Here, we investigated whether some AAS, as a result of a lack of target selectivity, might inhibit 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2)–dependent inactivation of glucocorticoids. Using recombinant human 11β-HSD2, we observed inhibitory effects for several AAS. Whereas oxymetholone, oxymesterone, danazol, and testosterone showed medium inhibitory potential, fluoxymesterone was a potent inhibitor of human 11β-HSD2 (half-maximal inhibitory concentration [IC50] of 60–100 nM in cell lysates; IC50 of 160 nM in intact SW-620, and 530 nM in MCF-7 cells). Measurements with rat kidney microsomes and lysates of cells expressing recombinant mouse 11β-HSD2 revealed much weaker inhibition by the AAS tested, indicating that the adverse effects of AAS-dependent 11β-HSD2 inhibition cannot be investigated in rats and mice. Furthermore, we provide evidence that fluoxymesterone is metabolized to 11-oxofluoxymesterone by human 11β-HSD2. Structural modeling revealed similar binding modes for fluoxymesterone and cortisol, supporting a competitive mode of inhibition of 11β-HSD2–dependent cortisol oxidation by this AAS. No direct modulation of mineralocorticoid receptor (MR) function was observed. Thus, 11β-HSD2 inhibition by fluoxymesterone may cause cortisol-induced MR activation, thereby leading to electrolyte disturbances and contributing to the development of hypertension and cardiovascular disease.

Key Words: anabolic androgenic steroid; fluoxymesterone; testosterone; 11β-hydroxysteroid dehydrogenase; hypertension; glucocorticoid; cardiovascular disease.

The misuse of anabolic androgenic steroids (AAS) represents a serious issue both in sports and in specific subsets of the population (Basaria, 2010). AAS are not only used as ergogenic drugs by athletes to enhance performance during competition but also for bodybuilding and for improving body image. Estimates suggested that the lifetime prevalence of AAS use in the United States is about 3% and that up to 6% of high school boys and 2% of girls use AAS (Basaria, 2010; Buckley et al., 1988; Hall, 2005; Kanayama et al., 2009; Yesalis et al., 1997). In addition to endogenously occurring steroids and synthetic AAS developed for clinical applications such as muscle wasting in human immunodeficiency virus infection and aging, an increasing number of designer steroids that have never been clinically tested often are present in dietary supplements, mostly without proper labeling (Kuhn, 2002; Parr and Schanzer, 2010).

The use of AAS has been associated with a series of adverse effects, including hepatotoxicity, psychological disturbances, virilization, suppressed spermatogenesis, gynecomastia, as well as premature mortality mainly as a result of cardiovascular complications (Handelsman, 2011; Parssinen et al., 2000). AAS exert direct effects on cardiomyocytes and vascular cells and indirect effects by decreasing high-density lipoprotein cholesterol and leading to early abnormalities in blood clotting factors (Ansell et al., 1993; Calof et al., 2005; Glazer, 1991). An impaired systolic and diastolic function has been directly associated with the duration and dose of AAS use in a recent study in power athletes (D’Andrea et al., 2007). Furthermore, AAS use has been associated with increased fluid retention and elevated blood pressure (Sullivan et al., 1998). Despite the recognition of the serious adverse cardiovascular effects of AAS misuse, the underlying molecular mechanisms remain largely unknown.

Impaired corticosteroid metabolism and activation of mineralocorticoid receptors (MR) have been associated with
cardiovascular disease (Briet and Schiffrin, 2010; Hadoke et al., 2009; Lastra et al., 2010). Two clinical hallmark studies supplementing the standard therapy (angiotensin-converting enzyme inhibitor, loop diuretic, and digoxin) with the MR antagonists spironolactone (Randomized Aldactone Evaluation Study, Pitt et al., 1999) or eplerenone (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study, Pitt et al., 2003b) revealed significantly reduced mortality in patients with myocardial infarction. Moreover, in patients with essential hypertension and left ventricular hypertrophy (4E-left ventricular hypertrophy study, Pitt et al., 2003a), the administration of the MR antagonist eplerenone lowered blood pressure and reduced left ventricular hypertrophy. Importantly, aldosterone levels were low in these patients, suggesting activation of MR by glucocorticoids, alternative ligands, or ligand-independent receptor activation (Frey et al., 2004; Odermatt and Atanasov, 2009).

Glucocorticoid-induced MR activation is especially relevant in situations of impaired activity of 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), which converts active 11β-hydroxycorticoids (cortisol and corticosterone) into their inactive 11-keto forms (cortisone and 11-dehydrocorticosterone) (Frey et al., 2004; Odermatt and Kratschmar, 2012). In addition to distal colon and renal cortical collecting ducts, coexpression of 11β-HSD2 and MR has been reported in the atria and the ventricles of the heart and in endothelial cells and vascular smooth muscle cells of the aorta (Caprio et al., 2008; Klusonova et al., 2009; Naray-Fejes-Toth and Fejes-Toth, 2007). Inhibition of 11β-HSD2 is expected to cause excessive cortisol-induced MR activation in these tissues. The impact of impaired 11β-HSD2 activity has been investigated inapolipoprotein E (−/−)/11β-HSD2 (−/−) double knockout mice where the loss of 11β-HSD2 stimulates inflammation and strikingly promotes atherosclerosis in an MR-dependent manner (Deuchar et al., 2011). In humans, genetic defects in the gene encoding 11β-HSD2 result in cortisol-induced MR activation and severe hypertension, ultimately leading to death by cardiovascular complications (Ferrari, 2010).

In the present study, we tested the hypothesis that some widely used AAS compounds might inhibit 11β-HSD2 and/or directly modulate MR activity.

MATERIALS AND METHODS

Materials. Methenolone was purchased from Cerilliant Corporation (Round Rock, TX), formebolone from LPB Instituto Farmaceutico S.p.A. (Cinisello, Rock, TX), fluphenazine from Steraloids (Newport, RI). The reagents and solvents used were of analytical and high performance liquid chromatography grade. [1,2,6,7-3H]-cortisol was purchased from PerkinElmer (Waltham, MA). All other chemicals were from Fluka AG (Buchs, Switzerland) of the highest grade available.

Cell culture. HEK-293 cells stably transfected with human 11β-HSD2 (Schweizer et al., 2003), human SW-620 colon carcinoma cells, and human MCF-7 breast cancer cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 4.5 g/l glucose, 50 U/ml penicillin/streptomycin, 2 mM glutamine, and 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4.

Determination of inhibition of human and mouse 11β-HSD2 using cell lysates. The conversion of cortisol to cortisone by 11β-HSD2 was measured as described previously (Gaware et al., 2011). Briefly, lysates of stably transfected HEK-293 cells were incubated for 10 min (human 11β-HSD2) or 20 min (mouse 11β-HSD2) at 37°C in a total volume of 22 μl of TS2 buffer (100 mM NaCl, 1 mM ethylene glycol tetraacetic acid [EGTA], 1 mM EDTA, 1 mM MgCl₂, 250 mM sucrose, and 20 mM Tris-HCl, pH 7.4) containing a final concentration of 200 nM cortisol, of which 10% was radiolabeled, 500 μM nicotinamide adenine dinucleotide (NAD⁺), and vehicle or inhibitor at the desired concentration. Reactions were terminated by adding methanol containing 2 mM unlabeled cortisol and cortisone, followed by separation of steroids by thin-layer chromatography (TLC) and analysis by scintillation counting. In addition, the inhibition of the conversion of cortisol to cortisone and fluoxymesterone to 11-oxofluoxymesterone by human 11β-HSD2 in cell lysates was assessed by liquid chromatography-mass spectrometry (for LC-MS method, see below). Lysates of HEK-293 cells stably expressing human 11β-HSD2 were incubated for 10 min at 37°C in a total volume of 500 μl at a final concentration of 200 nM cortisol, 500 μM NAD⁺, and various concentrations of fluoxymesterone. To assess inhibition of fluoxymesterone oxidation, lysates were incubated for 1 h at 37°C with 500 nM fluoxymesterone and various concentrations of cortisol. Reactions were stopped by shock freezing in a dry ice-ethanol bath. Concentrations of cortisol were low in these patients, suggesting activation of MR by glucocorticoids, alternative ligands, or ligand-independent receptor activation (Frey et al., 2004; Odermatt and Atanasov, 2009).
After thawing, samples were centrifuged at 4°C for 10 min at 500 × g and supernatants transferred onto a solid phase extraction column (Oasis HLB 1 cc [30 mg] Waters WAT094225) preconditioned with 1 ml of methanol and 1 ml of distilled water. After washing twice with 1 ml of water, steroids were eluted by twice adding 1 ml of methanol. The combined eluate was evaporated to dryness and reconstituted with 100 μl of methanol.

Steroids were resolved on an Atlantis T3 (3 μm, 2.1 × 150 mm) column (Waters, Milford, MA) at 30°C using an Agilent model 1200 Infinity Series chromatograph (Agilent Technologies, Basel, Switzerland). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). To separate fluoxymesterone and 11-oxofluoxymesterone, a linear gradient starting from 55% solvent A and 45% solvent B to 5% solvent A and 95% solvent B from 0 to 12 min was used. To separate cortisol and cortisone, a linear gradient starting from 85% solvent A and 15% solvent B to 60% solvent A and 40% solvent B from 0 to 15 min was used. The flow rate was maintained at 0.4 ml/min.

The LC was interfaced to an Agilent 6490 triple quad mass spectrometer (MS). The MS was operated in atmospheric pressure electrospray positive ionization mode, with nebulizer pressure and nebulizer gas flow rate of 45 psi and 10 l/h, respectively, a source temperature of 350°C and capillary and cone voltage of 4 kW and 190 V, respectively. Data acquisition was performed by Mass Hunter workstaton (Version B01.04). Metabolites were identified by comparing their retention time and mass to charge ratio (m/z) with those of authentic standards.

Qualitative determination of steroids was performed by selected positive ionization multiple reaction monitoring. Fluoxymesterone (m/z 377.1) and 11-oxofluoxymesterone (m/z 335.1) eluted at 9.1 and 9.7 min, respectively. For the quantitative determination of fluoxymesterone, prednisolone (m/z 361.1, retention time 7.3 min) was used as internal standard. Metabolites were quantified from calibration curves of the ratio of the peak area of the authentic fluoxymesterone standard and the internal prednisolone standard incubated with lysates of untransfected HEK-293 cells at a total protein concentration identical to that of the experimental setting and plotted against the concentration of authentic standards (normalization to the internal standard). A reference standard for 11-oxofluoxymesterone was not available and needs to be synthesized for confirmation of the structure and exact quantification. Cortisone (precursor and product ion at m/z 361 and 163) and cortisol (precursor and product ion at m/z 363 and 121) eluted at 8.5 and 8.9 min, respectively.

**Determination of the binding mode of steroids in 11β-HSD2 by molecular modeling.** The 2D structures of fluoxymesterone, testosterone, cortisone, and cortisol were generated using ChemBioDraw Ultra 12.0 (CambridgeSoft, U.K.; 1986–2009, www.cambridgesoft.com). Two-dimensional structures were transformed into 3D using CORINA (Molecular Networks GmbH, Erlangen, Germany). These four compounds were docked into the previously generated homology model of 11β-HSD2 (Gaware et al., 2011) using GOLD (Jones et al., 1997; Verdonk et al., 2003). The binding site was defined by a 10 Å sphere with the hydroxyl group of Tyr232 in the center. To optimize the ligand orientation, two protein hydrogen bond constraints were defined: one to the catalytically active Tyr232 and the second one to Tyr226. During the docking, the protein was handled as rigid and ligands as flexible. The ligand flexibility was increased by allowing the ring corner to flip. GoldScore was used as a scoring function, and 10 docking solutions for each ligand were calculated. Atom types for the protein as well as for the ligands were automatically determined by the program. LigandScout (Wolber and Langer, 2005) was used for visualizing the docking results and for the analysis of the protein-ligand interactions.

**RESULTS**

**Inhibition of 11β-HSD2 by Anabolic Androgenic Steroids Measured in Cell Lysates**

In order to test the hypothesis that AAS might cause adverse effects by disrupting corticosteroid hormone action, we tested several frequently used AAS for potential inhibition of the 11β-HSD2–dependent conversion of cortisol to cortisone as well as for their impact on MR transactivation. The activity of human 11β-HSD2 was assessed using lysates of stably transfected HEK-293 cells and by determining the conversion of radio-labeled cortisol to cortisone. At a high concentration (10μM), only norbolethone and methenolone did not have any inhibitory effect (Table 1). Dehydroepiandrosterone, mesterolone, and formebolone showed weak inhibitory effects with estimated Half-maximal inhibitory concentration (IC50) values of 10μM or higher. For the compounds that showed less than 40% remaining activity at this concentration, IC50 values were determined. This revealed potent inhibition by the 11β-hydroxylated steroid fluoxymesterone with IC50 of 60 ± 27nM and medium inhibitory effects for testosterone (Fig. 1A), oxymesterone, danazol, and oxymetholone. The inhibition of 11β-HSD2–mediated cortisol oxidation by fluoxymesterone was confirmed in an assay using unlabeled cortisol and quantification of the product cortisone by LC-MS. A slightly higher IC50 value of 100 ± 28nM was obtained (data not shown).

Adverse effects are usually studied in a next step in rodents. In preparing for animal studies, we tested whether fluoxymesterone would inhibit rat and mouse 11β-HSD2 in vitro. Measurements with rat kidney microsomes revealed an approximately 80-fold lower inhibitory potential with an IC50 of 4.9 ± 0.8μM (Fig. 1B). Similarly, an IC50 of 5.4 ± 0.4μM was obtained for fluoxymesterone inhibition of 11β-HSD2 using lysates of HEK-293 cells stably expressing the mouse enzyme (Fig. 1C). Testosterone was about 10-fold less potent toward the rat and the mouse enzymes with IC50 of 14.9 ± 4.3 and 15.3 ± 3.5μM, respectively. The weak

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining cortisol to cortisone conversion at 10μM (%)</th>
<th>IC50 (nM)</th>
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<tr>
<td>Glycyrrhetic acid</td>
<td>2.4 ± 1.8</td>
<td>256 ± 33</td>
</tr>
<tr>
<td>Testosterone</td>
<td>12 ± 12</td>
<td>1370 ± 60</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>78 ± 12b</td>
<td>nd</td>
</tr>
<tr>
<td>Danazol</td>
<td>33 ± 17</td>
<td>740 ± 110</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>1.9 ± 1.5</td>
<td>60 ± 27</td>
</tr>
<tr>
<td>Formebolone</td>
<td>51 ± 12</td>
<td>nd</td>
</tr>
<tr>
<td>Mesterolone</td>
<td>74 ± 6</td>
<td>nd</td>
</tr>
<tr>
<td>Methenolone</td>
<td>90 ± 3</td>
<td>nd</td>
</tr>
<tr>
<td>Norbolethone</td>
<td>92 ± 13</td>
<td>nd</td>
</tr>
<tr>
<td>Oxyxemesterone</td>
<td>2.6 ± 3.5</td>
<td>960 ± 140</td>
</tr>
<tr>
<td>Oxyxmetholone</td>
<td>6.4 ± 4.5</td>
<td>710 ± 50</td>
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Notes. The 11β-HSD2–dependent conversion of cortisol (50nM) to cortisone was measured in cell lysates in the presence of 500μM of NAD+ and various concentrations of AAS or vehicle (0.1% DMSO). Glycyrrhetic acid was included as reference compound. Inhibitory activities represent IC50 ± SD from at least three independent experiments, nd, not determined.

*Value taken from Kratschmar et al. (2011).

* Determined at 20μM.
inhibitory effects of fluoxymesterone and testosterone on the rodent 11β-HSD2 enzymes make it unlikely to study the adverse effects in these species, and animal experiments were not performed.

**Inhibition of 11β-HSD2 in Intact SW-620 and MCF-7 Cells**

The effect of fluoxymesterone on 11β-HSD2 activity was next determined in intact cells. Two distinct cell models, human colon carcinoma SW-620 cells and human breast cancer MCF-7 cells with relatively high endogenous expression of the enzyme were applied. Cell models with lower expression may not be suitable to determine direct effects on enzyme activity due to interference with altered gene expression. Fluoxymesterone potently inhibited 11β-HSD2 in a concentration-dependent manner with IC50 of 160 ± 10nM in SW-620 cells and IC50 of 530 ± 70nM in MCF-7 cells (Fig. 2). Although the known reference compound glycyrhretinic acid also showed potent inhibition, testosterone did not significantly inhibit 11β-HSD2 at concentrations up to 10μM in intact SW-620 or MCF-7 cells.

**Fluoxymesterone Does Not Directly Modulate MR Activation**

To see whether fluoxymesterone might directly modulate MR activity, transactivation assays in HEK-293 cells transiently expressing MR and a galactosidase reporter under the control of the mouse mammary tumor virus promoter were performed. Cells were incubated with 1μM of fluoxymesterone alone or together with 10nM aldosterone. The reporter gene response was indistinguishable if cells were treated with either aldosterone alone or in combination with fluoxymesterone (Fig. 3). Furthermore, basal receptor activity measured by DMSO treatment (0.05%) was not changed by fluoxymesterone. Similarly, the other tested AAS (see Table 1) did not activate or antagonize MR function, indicating that these steroids have no direct effect on the receptor.

**Fluoxymesterone Is Metabolized by 11β-HSD2**

Because fluoxymesterone possesses an 11β-hydroxyl group, we hypothesized that this steroid may be a substrate and therefore competitively inhibit the enzyme. Therefore, we established an LC-MS–based method using the multiple
reaction monitoring mode for the qualitative and quantitative determination of fluoxymesterone (m/z 337.1) and its metabolite 11-oxofluoxymesterone (m/z 335.1) (see Materials and Methods section). Fluoxymesterone and 11-oxofluoxymesterone were clearly separated with retention times of 9.1 and 9.7 min, respectively (Fig. 4). Similarly, conditions were established to separate and quantify cortisol (m/z 363, retention time 8.5 min) and cortisone (m/z 361, retention time 8.9 min). Upon incubation for 1 h with lysates of HEK-293 cells stably expressing 11β-HSD2, approximately 50% of the supplied fluoxymesterone (1 µM) was converted. In comparison, under the same conditions, approximately 80% of cortisol was converted. No conversion was observed when lysates of untransfected HEK-293 cells were used. Thus, although both steroids are substrates of 11β-HSD2 (Fig. 5), the enzyme more efficiently oxidizes cortisol.

To further support the hypothesis that fluoxymesterone and cortisol bind to and are metabolized by 11β-HSD2, we studied the impact of cortisol on the conversion of fluoxymesterone (at a final concentration of 500 nM) to 11-oxofluoxymesterone in cell lysates. A concentration-dependent inhibition with an IC₅₀ of 730 ± 190 nM was obtained (data not shown).

Comparison of the Binding Mode of Steroids in 11β-HSD2

All four compounds were docked into the 11β-HSD2 active site. They were all positioned in the vicinity of the catalytic triad Ser219-Tyr232-Lys236 (Fig. 6A). The binding interactions of each compound were investigated in detail to explain the difference in their activities. The automatically created pharmacophoric interaction pattern showed different hydrogen-bonding patterns for each compound (Figs. 6B–E).

The natural ligand cortisol was predicted to have favorable binding interactions. The 11β-hydroxy group interacts with the
catalytic residue Tyr232, and the ligand was anchored to the binding pocket with three other hydrogen bonds. The interaction pattern of cortisone, the end product of the reaction catalyzed by 11β-HSD2, differed to the one of cortisol in terms that the product moved the 11-keto group away from the catalytically active residues. The most active inhibitor, fluoxymesterone, was observed to be anchored to the binding pocket with four hydrogen bonds. The hydrogen bonds between Tyr232, Ser219,
and the 11-hydroxy group of fluoxymesterone indicated that this molecule can be transformed into its 11-keto form by the enzyme. In fact, biological data showed that fluoxymesterone is a substrate of 11β-HSD2, but with a lower conversion rate than cortisol. This lower conversion rate leads to a competitive inhibition of 11β-HSD2 by fluoxymesterone.

Interestingly, testosterone was predicted to adapt a different binding mode compared with the other three compounds. It was located in the same area as the others; however, it had a flipped binding mode. Whereas cortisol, cortisone, and fluoxymesterone had their 3-keto groups pointing toward Tyr226, the 3-keto group of testosterone superimposed onto position 17 of the other steroids. No other hydrogen bond between testosterone and the protein was observed. This kind of binding mode as well as fewer hydrogen bonds and hydrophobic interactions with the protein could be an explanation for the weaker 11β-HSD2 inhibition of testosterone.

**DISCUSSION**

In this study, we analyzed several well-known and widely used AAS for their potential to inhibit 11β-HSD2 and identified fluoxymesterone as a potent inhibitor. The IC_{50} values of 60–100nM in cell lysates and 160 and 530nM measured in intact SW-620 and MCF-7 cells, respectively, are in a comparable range with the inhibitory potential of glycyrhetinic acid, which has been demonstrated in both animals and humans to inhibit 11β-HSD2 and cause glucocorticoid-mediated MR activation, potassium excretion, sodium and water retention, and increased blood pressure (Ferrari, 2010; Ferrari et al., 2001; Serra et al., 2002). As shown for fluoxymesterone in the present study, glycyrhetinic acid also does not directly modulate MR function but competitively inhibits 11β-HSD2, thereby leading to glucocorticoid-mediated MR activation.

Oral fluoxymesterone, which is marketed as halotestin in the United States for the treatment of hypogonadism in men and of certain hormone-dependent mammary tumors in women, can exacerbate health states where edema plays important roles, such as congestive heart failure (as described in the package leaflet, [http://www.pfizer.com/files/products/uspi_halotestin.pdf](http://www.pfizer.com/files/products/uspi_halotestin.pdf)). A possible mechanistic explanation for these warnings concerning fluid retention states includes the involvement of MR activation. Following oral administration, fluoxymesterone is rapidly metabolized in the liver. The adverse effects described above can be expected to be much more pronounced upon intravenous application of the drug, which is the preferred route of administration in sports and bodybuilding.

Inhibition of 11β-HSD2 in the renal distal tubules and cortical collecting ducts results in glucocorticoid-induced MR activation with subsequently increased activities of Na\(^{+}/K\(^{-}\)-ATPases at the basolateral membrane and of Na\(^{+}\) channels (ENaC) on the apical membrane of epithelial cells, resulting in potassium excretion and sodium and water retention (Ferrari, 2010). In the vasculature, decreased 11β-HSD2 activity leads to vasoconstriction and elevated blood pressure, probably by a mechanism involving the activation of glucocorticoid receptor rather than MR (Goodwin et al., 2008). Furthermore, inhibition of 11β-HSD2 in vascular smooth muscle cells and endothelial cells of the heart may lead to paracrine activation of MR in cardiomyocytes. In a recent study using transgenic mice, Deuchar et al. (2011) showed that the ablation of 11β-HSD2 inapolipoprotein E knockout mice aggravated the progression of atherosclerosis by a mechanism involving MR activation and stimulation of inflammation in the vascular endothelium. The use of AAS has been associated with reduced levels of high-density lipoproteins and an increased ratio of low-density lipoproteins to high-density lipoproteins (Glazer, 1991; Thompson et al., 1989). Thus, AAS that inhibit 11β-HSD2, like fluoxymesterone, may be especially prone to cause adverse cardiovascular effects.

Regarding the inhibitory mechanism, both the 3D structural modeling and the fact that 11β-HSD2 can metabolize fluoxymesterone to 11-oxofluoxymesterone support a competitive inhibition of cortisol oxidation. The 11β-hydroxyl on fluoxymesterone has important stabilizing interactions that are absent in testosterone and other AAS. Interestingly, formebolone also has an 11β-hydroxyl but is a very weak 11β-HSD2 inhibitor, probably as a result of the methyl group in ring-A, which leads to steric hindrance and prevents optimal stabilization in the binding pocket. Although 11β-HSD2 can oxidize fluoxymesterone at position 11, this reaction represents a minor contribution to the overall metabolism of this steroid (Pozo et al., 2008). Compared with cortisol, fluoxymesterone was approximately three to five times less efficiently converted to its 11-oxo form, resembling the conversion of dexamethasone, which also has a fluor atom at position 9 (Rebuffat et al., 2004). A reference compound needs to be synthesized to test whether 11β-HSD2 might be able to reduce 11-oxofluoxymesterone as previously observed for 11-oxodexamethasone. This, together with the extensive hepatic metabolism, would explain why 11-oxo derivatives of fluoxymesterone are minor metabolites of fluoxymesterone in human urine (Pozo et al., 2008).

The potent inhibition of human but not rat and mouse 11β-HSD2 by fluoxymesterone emphasizes the importance to assess species-specific differences using recombinant enzymes and enzyme preparations from native tissues before conducting animal studies, both from an ethical point of view and to save resources. Clinical studies will need to investigate the potential of fluoxymesterone to inhibit 11β-HSD2 activity in vivo. This can be achieved by measuring its impact on the ratio of 11β-hydroxy- to 11-oxogluocorticoids in plasma and their corresponding tetrahydro metabolites in 24-h urine samples and by examining the patients for the presence of low-renin and low-aldosterone hypertension.
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