Hexose-6-phosphate dehydrogenase modulates the effect of inhibitors and alternative substrates of 11β-hydroxysteroid dehydrogenase 1

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Abstract

Intracellular glucocorticoid reactivation is catalyzed by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), which functions predominantly as a reductase in cells expressing hexose-6-phosphate dehydrogenase (H6PDH). We recently showed that the ratios of cortisone to cortisol and 7-keto- to 7-hydroxy-neurosteroids are regulated by 11β-HSD1 and very much depend on co-expression with H6PDH, providing cosubstrate NADPH. Here, we investigated the impact of H6PDH on the modulation of 11β-HSD1-dependent inter-conversion of cortisone and cortisol by inhibitors and alternative substrates. Using HEK-293 cells expressing 11β-HSD1 or co-expressing 11β-HSD1 and H6PDH, we observed significant differences of 11β-HSD1 inhibition by natural and pharmaceutical compounds as well as endogenous hormone metabolites. Furthermore, we show potent and dose-dependent inhibition of 11β-HSD1 by 7-keto-DHEA in differentiated human THP-1 macrophages and in HEK-293 cells over-expressing 11β-HSD1 with or without H6PDH. In contrast, 7-ketocholesterol (7-KC) did not inhibit 11β-HSD1 in HEK-293 cells, even in the presence of H6PDH, but inhibited 11β-HSD1 reductase activity in differentiated THP-1 macrophages ($IC_{50} = 8.1 \pm 0.9$ μM). 7-keto-DHEA but not 7-KC inhibited 11β-HSD1 in HEK-293 cell lysates. In conclusion, cellular factors such as H6PDH can significantly modulate the effect of inhibitors and alternative 7-oxygenated substrates on intracellular glucocorticoid availability.

Key words: 11β-hydroxysteroid dehydrogenase; glucocorticoid; 7-ketocholesterol; DHEA; oxysterol; hexose-6-phosphate dehydrogenase.

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; 7β-OHC, 7β-hydroxycholesterol; 7-KC, 7-ketocholesterol; DHEA, dehydroepiandrosterone; ER, endoplasmic reticulum; H6PDH, hexose-6-phosphate dehydrogenase; TLC, thin layer chromatography

Introduction

Glucocorticoid excess and locally disturbed glucocorticoid metabolism contribute to metabolic diseases (Atanasov and Odermatt, 2007). Understanding of the underlying mechanisms is indispensable for efficient treatment and prevention of these pathologies. In cells, glucocorticoids are activated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts cortisone to cortisol (or 11-dehydrocorticosterone to corticosterone in rodents). The major expression sites of 11β-HSD1 are liver, skeletal muscles and white adipose tissue, but it is also present in other organs such as brain and macrophages. In the latter, 11β-HSD1 can contribute to the immune response and increase the phagocytic capacity of macrophages (Gilmour et al., 2006).

Inside the cell, the enzyme is anchored in the endoplasmic reticulum (ER) membrane (Frick et al., 2004, Odermatt et al., 1999), with the catalytic site facing the lumen. The ER lumen is characterized by an oxidative environment with an estimated ratio of oxidized to reduced glutathione of 1:1-3 (Braakman et al., 1992). To act as a reductase, 11β-HSD1 (and probably other reductive enzymes inside the ER (Hori and Takahashi, 1974)) is dependent on the supply of NADPH. This can be achieved by hexose-6-phosphate dehydrogenase (H6PDH), which converts glucose-6-phosphate to 6-phosphogluconolactone and represents the ER-luminal analogue of the cytoplasmic glucose-6-phosphate dehydrogenase (Atanasov et al., 2004, Banhegyi et al., 2004, Bujalska et al., 2005). Moreover, direct interaction between 11β-HSD1 and H6PDH seems to be important for efficient reduction of cortisone (Atanasov et al., 2008). Importantly, mice lacking H6PDH are unable to convert 11-dehydrocorticosterone to corticosterone (Lavery et al., 2006).

Besides glucocorticoids, 11β-HSD1 binds some 7-oxygenated steroids and sterols in a rotated orientation and catalyzes their metabolism at position 7 (Nashev et al., 2007, Odermatt et al., 2006). Alternative 11β-HSD1 substrates include 7-ketocholesterol (7-KC) (Hult et al., 2004, Schweizer et al., 2004, Song et al., 1998), one of the major constituents of the atherosclerotic plaque, as well as 7-oxygenated dehydroepiandrosterone (DHEA) and pregnenolone metabolites (Muller et al., 2006, Nashev et al., 2007, Robinzon et al., 2003), which are important neurosteroids. Recently, we demonstrated that the direction of 11β-HSD1-catalyzed conversion of these substances very much depends on the presence of H6PDH in cellular systems (Nashev et al., 2007).

Here, we investigate the impact of H6PDH on the inhibitory potency of natural and pharmaceutical compounds as well as alternative substrates for 11β-HSD1, which may be relevant for efficacy and safety assessment of 11β-HSD1 inhibitors. Importantly, in some cells, *e.g.* in certain regions of the brain, 11β-HSD1 seems not to colocalize with H6PDH, as indicated by a recent expression study in the rat (Gomez-Sanchez et al., 2008). In addition, both 11β-HSD1 reductase and dehydrogenase activities have been measured in Leydig cells (Latif et al., 2005, Tomlinson et al., 2004), despite colocalization with H6PDH in these cells (Gomez-Sanchez et al., 2008). We used HEK-293 cells expressing 11β-HSD1 with or without H6PDH and measured the effect of inhibitors on the reduction of cortisone to cortisol in intact cells. In addition, we determined the impact of 7-keto and 7-hydroxy metabolites of cholesterol and DHEA on 11β-HSD1-dependent cortisone reduction in a relevant endogenous model, *i.e.* differentiated THP-1 macrophages. Our results show that H6PDH (and thus luminal NADPH availability) can influence the inhibitory potential of various compounds. Furthermore, we show that the alternative substrate 7-KC can inhibit 11β-HSD1 in differentiated THP1 macrophages.

To begin to understand catalysis of 7-KC by 11β-HSD1, we constructed 3D models of 11β-HSD1 and NADP(H) complexed with either 7-KC or 7β-hydroxycholesterol (7β-OHC), a method that we previously used to investigate how 11β-HSD1 catalyzes the formation of 7α-hydroxy- and 7β-hydroxy-DHEA from 7-keto-DHEA (Nashev et al., 2007). In that study, we found that 11β-HSD1 catalysis of 7-keto-DHEA to either 7α-hydroxy- or 7β-hydroxy-DHEA depended on whether the A ring or D ring of DHEA was oriented towards the interior of 11β-HSD1. Here, we report that analysis of the catalytic site of 3D models of 11β-HSD1 complexed

with NADP(H) and either 7-KC or 7β-OHC locates both steroids with their A ring oriented towards the interior of 11β-HSD1, which is the opposite orientation of glucocorticoids (Odermatt et al., 2006, Zhang et al., 2005). In our 3D model we find a favorable configuration of the catalytically important Tyr^{183} with 7-carbonyl on 7-KC but the distances between 7β-hydroxyl on 7β-OHC and the C4 atom on the nicotinamide ring of $NADP^+$ increases, which may explain the less efficient interconversion of 7-oxycholesterols compared with glucocorticoids by 11β-HSD1.

Materials and Methods

Materials. Cell culture media were purchased from Invitrogen (Carlsbad, CA) and Sigma (Buchs, Switzerland), [1,2,6,7-^{3}H]-cortisone from American Radiolabeled Chemicals (St. Louis, MO), [1,2,6,7-^{3}H]-cortisol from Amersham Pharmacia (Piscataway, NJ, USA), 5H-1,2,4-triazolo(4,3-a)azepine,6,7,8,9-tetrahydro-3-tricyclo(3·3·1·13·7)dec-1-yl (T0504) from Enamine (Kiev, Ukraine) and steroids and oxysterols from Steraloids (Wilton, NH). BNW7 and BNW16 were kindly provided by Dr. Thomas Wilckens, BioNetWorks GmbH (Munich, Germany). All other chemicals were from Fluka AG (Buchs, Switzerland) of the highest grade available.

Cell culture. HEK-293 cells stably transfected with human recombinant 11β-HSD1 (subsequently referred to as AT6) (Schweizer et al., 2003) or with both human 11β-HSD1 and human H6PDH (subsequently referred to as HHH7) (Gumy et al., manuscript submitted elsewhere) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 50 units/ml penicillin, 50 µg/ml streptomycin and 2 mM glutamine. THP-1 monocytes were generously provided by Dr. Brigitte Frey (University of Berne, Switzerland), and cultured in RPMI-1640 medium, supplemented with 10% FCS, 50 units/ml penicillin, 50 µg/ml streptomycin and 2 mM glutamine. Seventy-two hours prior to the experiment, cells were differentiated to macrophages by adding phorbol 12-myristate 13-acetate to the medium at a final concentration of 20 nM.

Determination of 11β-HSD1 activity. Enzymatic activities were determined in cell lysates and in intact cells as described previously (Nashev et al., 2007, Schuster et al., 2006). The reductase reaction was measured in a final volume of 20 µl containing 10 nCi [1,2,6,7-^{3}H]-cortisone, 200 nM unlabeled cortisone, 400 µM NADPH and various concentrations of inhibitors or steroid and sterol metabolites. Dehydrogenase activity was measured in the presence of 10 nCi radiolabeled

cortisol, 50 nM unlabeled cortisol and 400 μM $NADP^+$. For measurements in intact cells, 50'000 cells were seeded per well of poly-L-lysine coated 96-well Biocoat plates (Becton-Dickinson, Basel, Switzerland). The medium was replaced 24 h later by 80 μl fresh medium containing appropriate concentrations of inhibitors or steroid and sterol metabolites and 10 μl medium containing 10 nCi [1,2,6,7-^{3}H]-cortisone and 200 nM unlabeled cortisone. Cells were incubated for 30 min (HHH7) or 2 h (AT6) at 37°C, reactions stopped by adding an excess of unlabeled cortisone and cortisol in methanol, followed by separation of steroids by thin layer chromatography (TLC) and determination of the conversion of radiolabeled substrate by scintillation counting.

Differentiated THP-1 macrophages were incubated for 6 h with appropriate concentrations of 7-oxygenated sterols and steroids and activity assays were performed as described above. The remaining intact monolayers were washed twice with ice-cold PBS and lysed in 0.2 N NaOH containing 0.1% SDS (2 h, 4°C), and aliquots of the lysates were used to determine cellular protein content using the Pierce-BCA assay kit (Thermo Scientific, Rockford, IL, USA). Data (mean ± S.D.) were obtained from at least four independent experiments.

3D-Models of 11β-HSD1. Mouse 11β-HSD1 (PDB ID:1Y5R) was extracted from the Protein Data Bank (PDB) for use as a template to investigate the interactions of 7-KC and 7β-OHC with 11β-HSD1. We used 1Y5R because it contains both corticosterone and $NADP^+$, which allows us to superimpose C7-cholesterol analogs on corticosterone (Zhang et al., 2005). Cholesterol was extracted from PDB file 1N83. We converted cholesterol to 7-KC and 7β-OHC using the Biopolymer option in Insight II. Then we minimized the energy of each model of 11β-HSD1 with C7-cholesterol analogs using Discover 3, which was run for 10,000 iterations, using a distant dependent dielectric constant of 2.

Results and Discussion

Knowledge on the molecular mechanisms of the intracrine regulation of glucocorticoid homeostasis is essential to understand the mechanisms of various complex diseases, including the metabolic syndrome. The association of elevated glucocorticoid activation by 11β-HSD1 with metabolic diseases has stimulated efforts to identify selective inhibitors for this enzyme and to explore the consequences of systemic inhibition. Regulation of glucocorticoid action is highly dynamic and tissue-specific. The recent identification of H6PDH as a determinant for the function of 11β-HSD1 as a reductase (Atanasov et al., 2004, Banhegyi et al., 2004, Bujalska et

al., 2005) and evidence from expression analyses of the two enzymes (Gomez-Sanchez et al., 2008) indicate significant tissue-specific regulation of glucocorticoid reactivation by 11β-HSD1. Moreover, 11β-HSD1 plays a role in the metabolism of 7-KC (Hult et al., 2004, Schweizer et al., 2004, Song et al., 1998) and 7-oxygenated neurosteroids (Muller et al., 2006, Nashev et al., 2007, Robinzon et al., 2003). These so-called alternative functions suggest a role for 11β-HSD1 in detoxification reactions which might be beneficial, further highlighting the importance of studying the tissue-specific functions of 11β-HSD1 and the relevance of H6PDH. Therefore, we investigated the influence of H6PDH on the effects of inhibitors and alternative substrates on 11β-HSD1-dependent conversion of cortisone to cortisol.

Impact of H6PDH on effects of 11β-HSD1 inhibitors in intact cells

Because the substrate to cosubstrate ratio seems to have a major impact on 11β-HSD1 activity (and thus on its inhibition) (Castro et al., 2007, Dzyakanchuk et al., 2008), we hypothesized that the presence or absence of H6PDH influences the potency of 11β-HSD1 inhibitors, particularly of own endogenous substrates (Table 1).

Table 1. Inhibition of human 11β-HSD1 dehydrogenase and reductase activities in cell lysates. 11β-HSD activities were determined in lysates of HEK-293 cells expressing the corresponding human recombinant enzyme as described in "Materials and Methods". Data represent IC_{50} values in μM (mean ± S.D.) from four independent experiments. *n. d.* = not detectable.

Compound	11β-HSD1 oxidation	11β-HSD1 reduction
Glycyrrhetinic acid	0.23 ± 0.02	0.40 ± 0.08[a]
Abietic acid	1.90 ± 0.38	5.4 ± 0.4[a]
Flavanone	n. d.	21 ± 3[a]
2'-Hydroxyflavanone	37 ± 5	24 ± 3[a]
T0504	0.041 ± 0.011	0.021 ± 0.003[a]
BNW7	n. d.	2.0 ± 0.2[b]
BNW16	0.39 ± 0.08	0.144 ± 0.027[b]
7-ketodehydroepiandrosterone	29 ± 5[c]	0.82 ± 0.07[c]
7α-hydroxydehydroepiandrosterone	16 ± 1[c]	45 ± 2[c]
7β-hydroxydehydroepiandrosterone	0.54 ± 0.05[c]	7.7 ± 0.8[c]
7-ketopregnenolone	5.4 ± 0.5[c]	0.68 ± 0.11[c]
7β-hydroxypregnenolone	1.34 ± 0.49[c]	2.4 ± 0.5[c]
5α-androstan-3β-ol-7,17dione	18 ± 1[c]	0.50 ± 0.11[c]

Values are from [a](Arampatzis et al., 2005); [b](Schuster et al., 2006) and [c](Nashev et al., 2007)

Therefore, we measured 11β-HSD1 activity in HEK-293 cells stably expressing 11β-HSD1 alone or together with H6PDH. As shown in Table 2, we found that in the presence of H6PDH, the required inhibitory concentration of many compounds underwent significant changes.

Table 2. Inhibition of human 11β-HSD1 in the absence or presence of H6PDH measured in intact cells. The reduction of cortisone by 11β-HSD1 was determined in intact HEK-293 cells expressing recombinant enzyme as described in "Materials and Methods". Data represent IC_{50} values in μM and are mean ± S.D. from four independent experiments.

Compound	11β-HSD1	11β-HSD1 H6PDH
Glycyrrhetinic acid	0.95 ± 0.32	0.55 ± 0.14
Abietic acid	14.4 ± 2.3	7.5 ± 0.74
Flavanone	0.51 ± 0.15	4.0 ± 1.2
2'-Hydroxyflavanone	2.5 ± 0.6	2.7 ± 0.7
T0504	0.139 ± 0.023	0.015 ± 0.001
BNW7	0.54 ± 0.05	5.2 ± 0.7
BNW16	2.3 ± 0.2	0.41 ± 0.08
7-ketodehydroepiandrosterone	0.37 ± 0.12	0.42 ± 0.05
7α-hydroxydehydroepiandrosterone	stimulation	105 ± 10
7β-hydroxydehydroepiandrosterone	stimulation	25 ± 4
7-ketopregnenolone	0.38 ± 0.09	0.151 ± 0.016
7β-hydroxypregnenolone	stimulation	6.4 ± 0.1
5α-androstan-3β-ol-7,17dione	0.144 ± 0.017	0.45 ± 0.02

For flavanone, a potent inhibitor of 11β-HSD1 reductase activity in intact cells not expressing H6PDH, eight times higher concentrations were required to obtain comparable inhibition rates in the presence of H6PDH. In cell lysates, flavanone did not inhibit the dehydrogenase reaction and was a relatively weak inhibitor of the reductase activity (Table 1). In contrast, the IC_{50} values of 2'-hydroxyflavanone were similar in 11β-HSD1 *versus* 11β-HSD1 and H6PDH expressing intact cells, and 2'-hydroxyflavanone inhibited both dehydrogenase and reductase activities of 11β-HSD1 in cell lysates, although at relatively high concentrations (Table 1). The synthetic compound T0504 (also known as Merck-544) displayed a ten-fold higher potency in the presence of H6PDH than in its absence (Table 2). The pharmaceutical compounds BNW7 and BNW16 also showed differences in their inhibitory potential upon coexpression with H6PDH.

The IC_{50} for 11β-HSD1 of BNW7 was 10-fold higher and that of BNW16 approximately 6-fold lower upon coexpression with H6PDH.

The 7-keto steroids 7-keto-DHEA, 7-ketopregnenolone and 5α-androstane-3β-ol-7,17-dione, which are substrates for the 11β-HSD1 reductase activity (Nashev et al., 2007), showed comparable IC_{50} values in cells expressing 11β-HSD1 alone or together with H6PDH. In lysates, they showed a strong preference to inhibit the reductase activity of 11β-HSD1. In contrast, the corresponding 7-hydroxy metabolites preferentially inhibited the dehydrogenase activity of 11β-HSD1 in lysates, whereas they were weak inhibitors of the reductase activity in intact cells expressing H6PDH, and even showed a concentration-dependent stimulation of the reductase activity at lower concentrations in the absence of H6PDH (Fig. 1).

This observation remains an enigma. A possible explanation is that inhibition of the dehydrogenase activity of the reversible 11β-HSD1 enzyme by the 7β-hydroxy metabolites results in a higher net conversion of cortisone to cortisol. In the presence of H6PDH, the reductase reaction is predominant *in vivo* and inhibition of the dehydrogenase reaction by 7β-hydroxysteroids has a negligible effect. However, the same explanation is not valid for the reverse reaction, because no stimulation of the dehydrogenase activity could be observed in the presence of 7-ketosteroids (data not shown), suggesting differences in the sequential binding mechanism between the reductase and dehydrogenase reaction. Castro *et al.* suggested that the inhibitory potency of carbenoxolone is strongly influenced by the presence of NADPH and substrate in the assay buffer. The apparent K_i values for carbenoxolone decreased at high concentrations of NADPH, suggesting that carbenoxolone behaved as competitive inhibitor against cortisone and as noncompetitive inhibitor against NADPH (Castro et al., 2007). Nevertheless, the kinetic parameters of each inhibitor need to be examined individually, because the presence of sufficient amount of NADPH, the substrate: cofactor: inhibitor ratio and the mode of inhibition may provide an explanation for the observed differences.

Figure 1

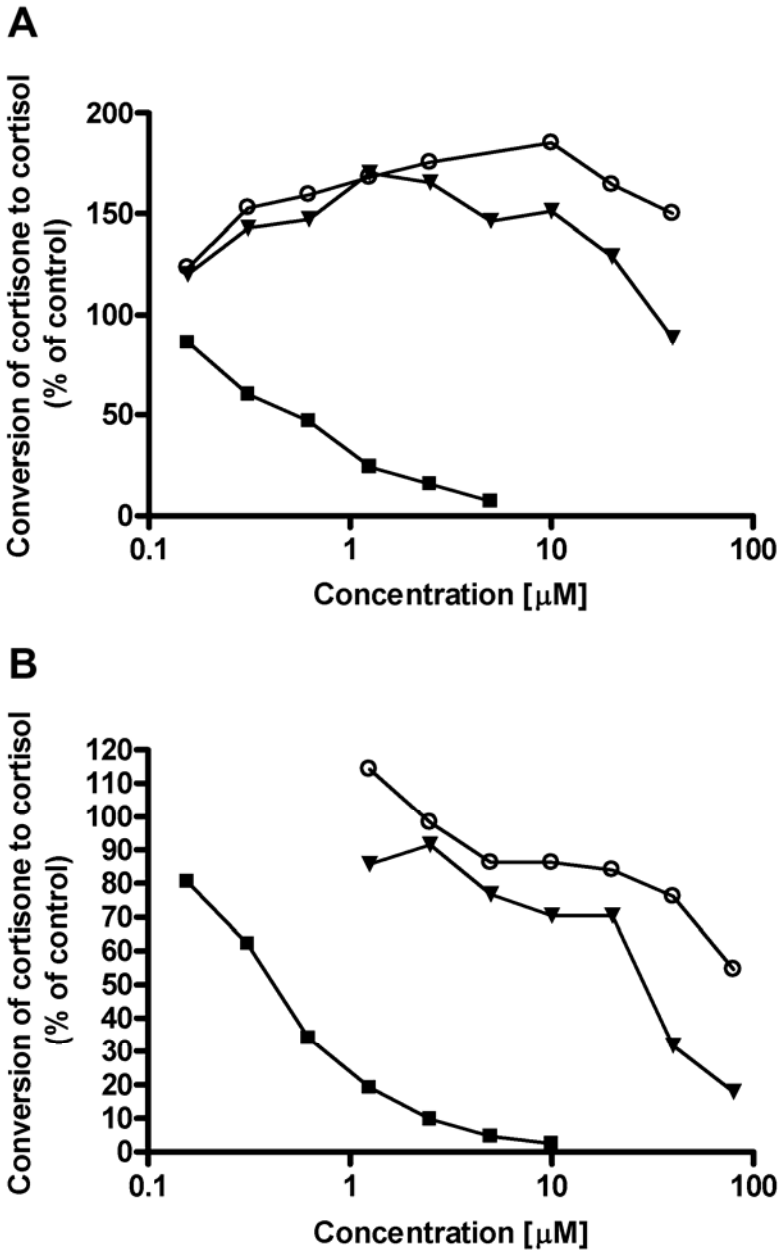

Figure 1. Effect of 7-oxygenated DHEA metabolites on 11β-HSD1 dehydrogenase activity in HEK cells. HEK-293 cells stably expressing human 11β-HSD1 (AT6, *A*) or human 11β-HSD1 and human H6PDH (HHH7, *B*) were incubated with 200 nM cortisone for 2 h (*A*) or 30 min (*B*) in the presence of 7-oxygenated steroids. Enzyme activity was determined as described in "Materials and Methods". One representative experiment out of four is shown. Data represent % conversion, whereby the activity of control cells incubated with vehicle was set to 100%. 7keto-DHEA (■), 7α-DHEA (○), 7β-DHEA (▼).

11β-HSD1 activity is inhibited by its substrate 7-KC in THP-1 macrophages but not in AT6 and HHH7 cells

The oxidized cholesterol metabolite 7-KC is a biologically relevant compound. It is one of the major oxysterols accumulated in foam cells of the atherosclerotic plaque and is enriched also in cholesterol containing processed (*i.e.* heat-treated) food (Brown and Jessup, 1999, Gill et al., 2008, Schroepfer, 2000). Recently, we and others showed that 11β-HSD1 is able to catalyze the interconversion of 7-KC and 7β-hydroxycholesterol in cellular systems and in the liver of the rat (Arampatzis et al., 2005, Hult et al., 2004, Schweizer et al., 2004). Based on the fact that 11β-HSD1 accepts both cortisone and 7-KC as substrates with comparable kinetic properties, we expected that 7-KC acts as a potent competitive inhibitor of the 11β-HSD1 reductase activity. Surprisingly, we could not detect any inhibition of the enzyme in cell lysates or intact HEK-293 cells stably expressing 11β-HSD1 or both 11β-HSD1 and H6PDH, even when 7-KC was supplied at high concentrations (Fig. 2A). In contrast, incubation of differentiated THP-1 macrophages with 7-KC resulted in significantly decreased 11β-HSD1 activity (IC_{50} 8.1 ± 0.9 μM; Fig. 2A). 7-KC did not show cellular toxicity at the conditions applied as assessed by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and measurement of cellular protein (data not shown). 7β-OHC was also able to inhibit 11β-HSD1 reductase activity in THP-1 macrophage, although about half as efficiently as 7-KC, but not in HEK cells. Moreover, during revision of this manuscript a paper by Wamil et al. reported reduction of 7-KC by 11β-HSD1 and inhibition of the reduction of cortisone by 7-KC in 3T3-L1 adipocytes (Wamil et al., 2008). Why 7-KC and 7β-OHC inhibit 11β-HSD1 in THP-1 macrophages and 3T3-L1 adipocytes but not in HEK cells is currently unclear, especially since 7-keto-DHEA and 7-ketopregnenolone resulted in potent inhibition with comparable IC_{50} values both in macrophages and HEK cells (Fig. 2A). 7-hydroxy-DHEA metabolites were also weak inhibitors of 11β-HSD1 reductase activity in differentiated THP-1 macrophages (Fig. 2B). Possible explanations for the cell-specific effect of 7-KC on 11β-HSD1 include differential metabolism of 7-KC (see below), differential post-translational modification of 11β-HSD1 and/or differences in the intracellular accumulation of oxysterols.

Figure 2

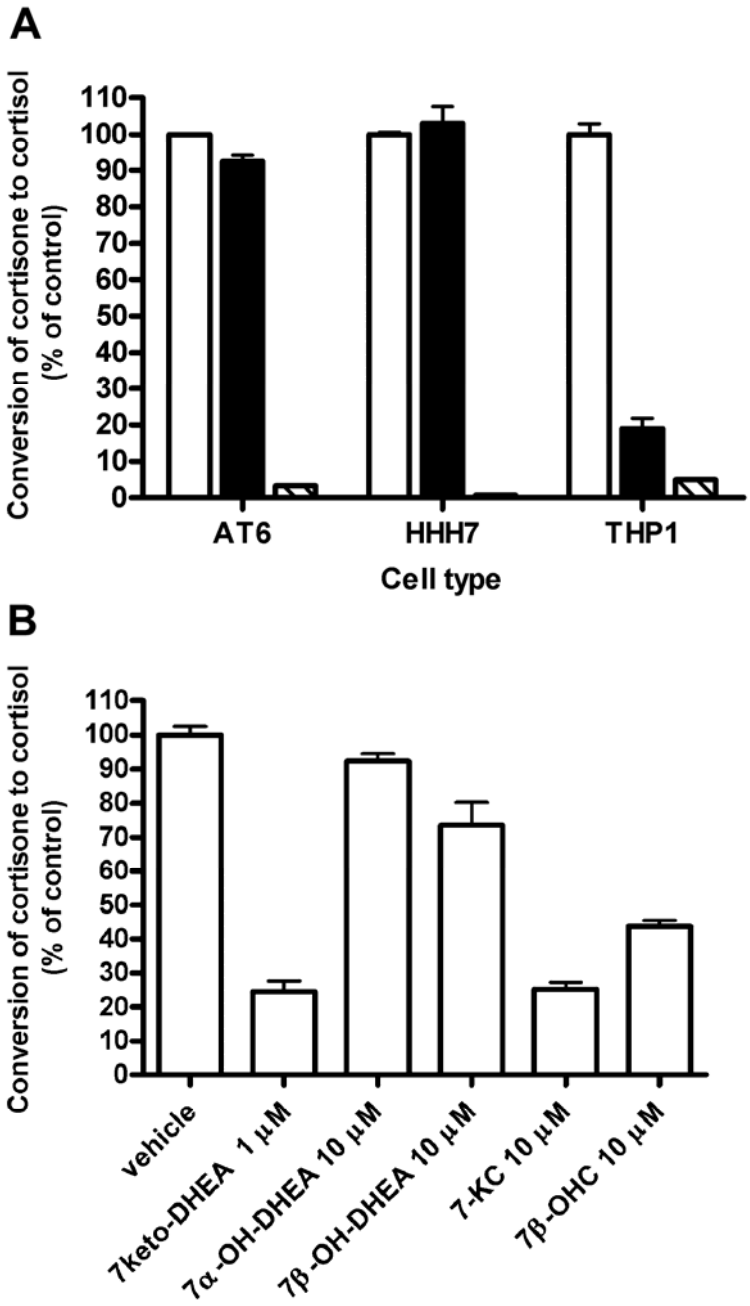

Figure 2. Inhibition of 11β-HSD1 by 7-oxygenated DHEA and cholesterol metabolites. **A.** Inhibition of 11β-HSD1 in differentiated THP-1 macrophages but not in HEK cells. Cells were incubated with 7-KC (20 μM) or 7-keto-DHEA (20 μM) and radiolabeled cortisone (200 nM) for 30 min (HHH7), 2 h (AT6) or 6 h (THP-1). Enzyme activity was determined as described in "Materials and Methods". Data are expressed as percentage of the conversion

detected with cells incubated with vehicle. Open bars, vehicle; filled bars, 7-KC 20 μM; hatched bars, 7-keto-DHEA 20μM.
B. Effect of 7-oxygenated DHEA and cholesterol metabolites on 11β-HSD1 reductase activity in differentiated THP-1 macrophages. Differentiated THP-1 macrophages were incubated with 1 μM 7-keto-DHEA, 10 μM 7α-hydroxy-DHEA, 10 μM 7β-hydroxy-DHEA, 10 μM 7-KC or 10 μM 7β-OHC in medium containing radiolabeled cortisone (200 nM) for 6 h. Enzyme activity was measured as described in "Materials and Methods".

Analysis of 7-oxycholesterol binding to 11β-HSD1 by 3D-modelling

Our data and those of others show that 11β-HSD1 catalyzes the formation of 7β-OHC from 7-KC (Hult et al., 2004, Schweizer et al., 2004, Wamil et al., 2008). Based on our previous analysis (Nashev et al., 2007), this indicates that during catalysis 7-KC has the A ring oriented towards the interior of 11β-HSD1, which is opposite of that for corticosterone (Fig. 3A)(Zhang et al., 2005). Thus, we modeled 7-KC and 7β-OHC in 11β-HSD1 with their A ring oriented towards the interior of 11β-HSD1 as shown in Figure 3B and 3C. In each panel in Figure 3, we show the interactions of Ser^{170} and Tyr^{183} with the steroid substrates, Tyr^{183} with NADPH and Lys^{187} with the ribose hydroxyls. These are highly conserved interactions in SDRs (Jornvall et al., 1995, Sciotti et al., 2006).

As reported by Zhang et al., Ser^{170} and Tyr^{183} on 11β-HSD1 are 3.1 Å and 2.8 Å, respectively, from the C11-hydroxyl on corticosterone (Zhang et al., 2005). The C11 hydroxyl is 3.9 Å from the C4 atom on the nicotinamide ring on NADPH. Lys^{187} is 3.2 Å from each ribose hydroxyl on NADPH (Fig. 3A).

Similar distances are found in the 11β-HSD1/NADPH 7-KC complex (Fig. 3B). Ser^{170} and Tyr^{183} are 2.8 Å from the C7-ketone on 7-KC. Tyr^{183} is 3.8 Å from the nicotinamide C4. Lys^{187} is 3.6 Å from each ribose hydroxyl, which is a favorable distance, although it is a weaker hydrogen bond than is found in the 11β-HSD1 complex with corticosterone (Fig. 3A).
In the 11β-HSD1/NADPH/7β-OHC complex, Ser^{170} and Tyr^{183} are 2.8 Å and 3.8 Å, respectively, from the 7β-hydroxyl (Fig. 3C). There are, however, two distances in the complex which would be expected to reduce catalysis of 7β-OHC by 11β-HSD1. First, the 7β-hydroxyl is 4.5 Å from the nicotinamide C4. Second, the Lys^{187} side chain has rotated so that it is 5.1 Å from the NO3 ribose hydroxyl. The distances of these latter two key interactions in the catalytic site of the 11β-HSD1/NADPH/7β-OHC complex suggest a suboptimal interaction of 7β-OHC with

11β-HSD1 which may explain the less efficient catalysis by 11β-HSD1 of 7β-OHC compared with that of 11β-hydroxyglucocorticoids.

Figure 3

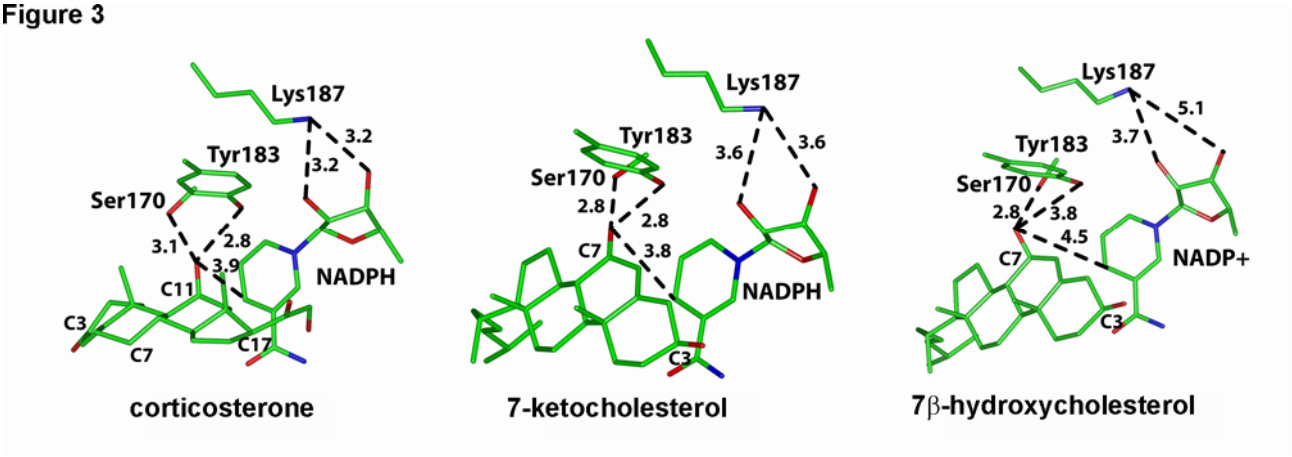

Figure 3. 3D models of 7-KC and 7β-OHC in mouse 11β-HSD1.
A. 11β-HSD1 complexed with corticosterone and NADPH (Zhang et al., 2005). In the crystal structure of 11β-HSD1, the D ring of corticosterone is orientated towards the interior of 11β-HSD1. The catalytically active Ser^{170} and Tyr^{183} and the nicotinamide C4 are favorably positioned to interact with the 11β-hydroxyl on corticosterone. Lys^{187} is 3.2 Å from each ribose hydroxyl on NADPH.
B. 11β-HSD1 complexed with 7-KC and NADPH. The A ring is orientated towards the interior of 11β-HSD1. As a result, the C7 and C11 positions on 7-KC flip so that the C7-ketone has favorable interactions with Ser^{170}, Tyr^{183} and the nicotinamide C4. Lys^{187} is 3.6 Å from each ribose hydroxyl.
C. 11β-HSD1 complexed with 7β-OHC and NADPH. The A ring is orientated towards the interior of 11β-HSD1. The 7β-hydroxyl on 7β-OHC is 2.8 Å and 3.8 Å, respectively, from Ser^{170} and Tyr^{183}. The 7β-hydroxyl is 4.5 Å from the nicotinamide C4, and Lys^{187} has rotated so that it is 5.1 Å from the NO3 ribose hydroxyl. These two unfavorable distances may contribute to suboptimal binding of 7β-OHC to 11β-HSD1.

Also of importance for substrate binding and catalysis is the different orientation in 11β-HSD1 of both cholesterol molecules compared with that of corticosterone. The interaction of 11β-HSD1 with the corticosterone D ring, which has a C17 side chain containing a ketone at C20 and alcohol at C21, will be different than that for the A ring of two cholesterol analogs, which have a 3β-hydroxyl. These differences are likely to influence enzyme kinetics and may explain the absence of 7-OHC catalysis by 11β-HSD1 in intact HEK293 cells as due to post-translational modifications, such as phosphorylation or glycosylation. Such post-translational

modifications of 11β-HSD1 would not affect binding or catalysis of glucocorticoids, and indeed, may even optimize catalytic efficiency, but they may interfere with binding of 7-oxycholesterols due to the different orientation in 11β-HSD1.

Potential relevance of the 11β-HSD1-dependent metabolism of 7-KC

11β-HSD1 preferentially converts 7-KC to 7β-OHC in the liver, where it may play a role in the detoxification of food-derived 7-KC by catalyzing the first step toward the formation of bile acids and subsequent excretion (Schweizer et al., 2004). 11β-HSD1-dependent metabolism of 7-KC might also be relevant in macrophages in the pathogenesis of atherosclerosis. It has been suggested that 7-KC is toxic and pro-atherogenic, for example by inhibiting cholesterol efflux from foam cells, inducing apoptosis and altering endothelial permeability (Brown and Jessup, 1999, Jessup and Brown, 2005). The relative ratio of different oxysterol metabolites seems to be important for the final (patho)physiological output (Biasi et al., 2004, Steffen et al., 2006). Our efforts to elucidate 11β-HSD1-dependent 7-KC metabolism in macrophages have not yet succeeded, given the disappearance of added 7-KC without formation of significant amounts of 7β-OHC (data not shown). This may be due to rapid subsequent metabolism of 7β-OHC formed by 11β-HSD1 to an unknown product; alternatively, the 11β-HSD1-mediated pathway may not be the exclusive route of 7-KC metabolism in macrophages (Jessup and Brown, 2005). Several recent reports suggest 27-hydroxylase as an important enzyme responsible for 7-KC metabolism, resulting in the formation of 7-keto-27-hydroxycholesterol (Brown et al., 2000, Lyons and Brown, 2001). This hypothesis, however, could not be confirmed in 27-hydroxylase deficient mice (Lyons et al., 2002), which showed enhanced hepatic metabolism of 7-KC. Nevertheless, the high concentration of 7-KC in macrophages of atherosclerotic plaques is expected to interfere with 11β-HSD1-dependent glucocorticoid activation and further studies are required to elucidate the relevance of 11β-HSD1 function in macrophages.

Conclusions

The present study emphasizes the importance of considering H6PDH in the analysis of the properties of 11β-HSD1 inhibitors. H6PDH can have a significant influence on the potency of pharmaceutical or natural compounds designed to decrease 11β-HSD1-dependent generation

of active glucocorticoids. Moreover, tissue-specific expression of H6PDH allows fine-tuned regulation of the 11β-HSD1-mediated interconversion both of glucocorticoids and of 7-oxygenated steroids and sterols. It will be important to investigate the potential interference of systemically applied 11β-HSD1 inhibitors with these alternative (i.e. non-glucocorticoid) activities as well as that of 11β-HSD1 dehydrogenase, which may not be present in some tissues including brain and testis.

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