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11β-Hydroxysteroid dehydrogenase type 1: Purification from human liver and characterization as carbonyl reductase of xenobiotics

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Abstract

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the interconversion of 11-oxo glucocorticoids to their 11-hydroxy metabolites, thereby controlling access of glucocorticoid hormones to the glucocorticoid receptor. Interestingly, evidence is emerging that 11β-HSD1 fulfills an additional role in the metabolism of xenobiotic carbonyl compounds. In our studies, 11β-HSD1 was identified as a microsomal reductase that initiates the final detoxification of xenobiotics by reducing them to alcohols that are easier to conjugate and eliminate. With its pluripotent substrate specificities for glucocorticoids and xenobiotics, 11β-HSD1 adds to an expanding list of those hydroxysteroid dehydrogenases which, on the one hand, are capable of catalyzing the carbonyl reduction of non-steroidal carbonyl compounds, and which, on the other hand, exhibit great specificity to their physiological steroid substrates. It is conceivable that large interferences must occur between endogenous steroid metabolism and the detoxification of xenobiotic compounds on the level of hydroxysteroid dehydrogenases.

Keywords: 11β-Hydroxysteroid dehydrogenase; Glucocorticoid metabolism; Enzyme cooperativity; Carbonyl reduction; Smoking; NNK; Detoxification; Licorice

1. Introduction

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a microsomal enzyme that catalyzes the reversible interconversion of receptor-active 11-hydroxy glucocorticoids (cortisol) to their receptor-inactive 11-oxo metabolites (cortisone). However, the physiological role of 11β-HSD1 as pre-receptor control device in regulating access of glucocorticoid hormones to the glucocorticoid receptor remains obscure in light of its low substrate affinities, which is in contrast to low glucocorticoid plasma levels and low K_d values of the receptors to cortisol. To solve this enigma, we performed detailed kinetic analyses with a homogenously purified 11β-HSD1 from human liver. The membrane-bound enzyme was successfully obtained in an active state by a purification procedure that took advantage of a gentle solubilization method as well as providing a favourable detergent surrounding during the various chromatographic steps (Maser et al., 2002). The identity of purified 11β-HSD1 was proven by determination of enzymatic activity, N-terminal amino acid sequencing and immunoblot analysis. Interestingly, 11β-HSD1 is active as a dimeric enzyme and exhibits Michaelis–Menten kinetics with cortisol and corticosterone (11β-dehydrogenation activity), but cooperative kinetics with cortisone and dehydrocorticosterone (11-oxoreducing activity). Accordingly, this enzyme provides the fine tuning of cortisol supply to the glucocorticoid receptor required as a consequence of great variations in circadian plasma glucocorticoid levels.

Evidence is emerging that 11β-HSD1 fulfills an additional role in the detoxification of non-steroidal carbonyl compounds, by catalyzing their reduction to the corresponding hydroxy derivatives that are easier to conjugate and eliminate (Maser and Oppermann, 1997). Due to its cooperativity for carbonyl reduction, 11β-HSD1 is also able to metabolize even nanomolar concentrations of the tobacco-specific nitrosamine 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK), a fact which is important in view of the relatively low levels of this carcinogen observed in smokers (Maser et al., 2003). 11β-HSD1 is potently (in nanomolar concentrations) inhibited by glycyrrhetinic acid, the main constituent of licorice (Maser et al., 2003). Hence, licorice exposure may affect NNK detoxification by inhibition of 11β-HSD1 (Maser, 2004). Collectively, our data expand insights into the multifunctional nature of hydroxysteroid dehydrogenases/carbonyl reductases and emphasize the importance of 11β-HSD1 in the detoxification of a tobacco-derived carcinogen, in addition to its endocrinological functions.

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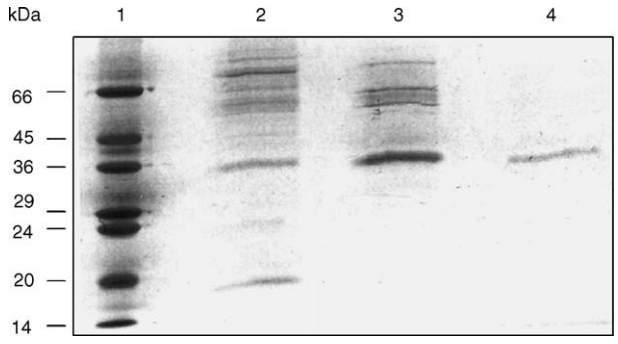

Fig. 1. Purification of 11β-HSD1 from human liver microsomes. 11β-HSD1 was successfully obtained in an active state by a purification procedure that took advantage of a gentle solubilization method as well as providing a favourable detergent surrounding during the various chromatographic steps. The purification yielded an active enzyme with a single band of 34 kDa on an SDS-polyacrylamide gel. Lane assignments are as follows: 1, standard; 2, octyl-Sepharose; 3, Mono Q-Sepharose; 4, red Sepharose A.

2. Purification of 11β-HSD1 from human liver

Although being membrane-bound to the endoplasmic reticulum and remarkably sensitive to purification, 11β-HSD1 could successfully be purified in an active state from human liver (Maser et al., 2002). The total quantity of microsomal protein was progressively decreased by the chromatographic purification procedure. The last step yielded a single band in the 34 kDa region as compared to the standard, showing the 11β-HSD1 monomer and giving evidence that the protein was purified to homogeneity (Fig. 1).

3. 11β-HSD1 is active as a dimer

Gel-permeation chromatography revealed that 11β-HSD1 is active as a dimer in vivo and that it can be purified from human liver retaining its functional properties like those 11β-HSD1 forms in intact tissues (Maser et al., 2002) (Fig. 2).

4. Cooperativity of 11β-HSD1 upon glucocorticoid reduction

The kinetic data indicate that both 11-dehydrogenation and 11-oxoreduction of glucocorticoids can be catalyzed by purified 11β-HSD1 from human liver (Table 1). Further, when Michaelis–Menten kinetics were considered, the K_m values for glucocorticoid oxidoreduction range in low micromolar concentrations, thus corresponding to literature results obtained from crude microsomal fractions (Agarwal et al., 1990; Moore et al., 1993; Diederich et al., 1998; Pu and Yang, 2000). However, when applying the sigmoidal dose–response kinetics, it was somewhat surprising to observe cooperative kinetics of 11β-HSD1 with cortisone and dehydrocorticosterone (glucocorticoid 11-oxo-reducing activity) with Hill-coefficients of 1.6 and 2.7, respectively (Maser et al., 2002) (Fig. 3). Accordingly, the 11β-HSD1 enzyme is obviously able to adapt to low (nanomolar) as well as to high (micromolar) substrate concentrations. The fact that this enzyme cooperativity was not observed for 11-dehydrogenation further emphasizes the physiological role of 11β-HSD1 to act mainly as glucocorticoid reductase.

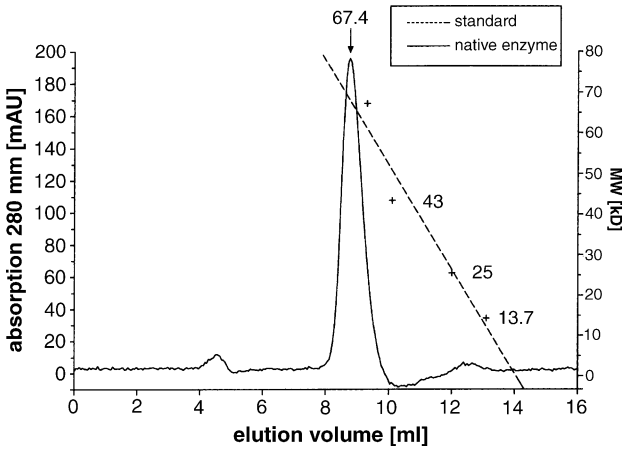

Fig. 2. Evidence for the dimeric nature of 11β-HSD1. Gel filtration of purified 11β-HSD1 was performed on a Pharmacia ÄKTA Protein Purifier system with a Superdex75 column (HiLoad 26/60). 11β-HSD1 eluted exclusively at 67.4 kDa as an active enzyme (shown by an arrow), thus revealing the dimeric nature of 11β-HSD1 from human liver (Maser et al., 2002). Molecular mass markers (kDa): 13.7, ribonuclease A; 25, chymotrypsinogen; 43, ovalbumin; 67, bovine serum albumin.

5. 11β-HSD1 acts as carbonyl reductase for xenobiotics

5.1. 11β-HSD1 initiates the detoxification of the tobacco-specific carcinogen NNK

NNK is the most potent carcinogenic agent contained in cigarette smoke and has been suggested to be an important etiological factor in tobacco-smoke related human cancer (Hecht, 1998). The genotoxicity of NNK is dependent on the relationship between its metabolic activation by cytochrome P450 enzymes and its final detoxification by carbonyl reduction to NNK alcohol (NNAL) followed by glucuronidation (Hecht, 1998) (Fig. 4).

In previous investigations, 11β-HSD1 was identified as a microsomal NNK carbonyl reductase that initiates the final

Table 1
Kinetic constants of purified 11β-HSD1

Substrate	V_{max} (nmol min^{-1} mg^{-1})	K_m (μM)	V_{max}/K_m	Hill-coefficient
Cortisone	5.3	13.9	0.38	1.6
Cortisol	19.0	41.3	0.46	1.0
Dehydrocorticosterone	4.0	19.7	0.20	2.7
Corticosterone	10.6	42.8	0.25	0.7
Oracin[a]	0.84	190	0.004	2.3
Oracin[b]	2.5	150	0.017	1.8
NNK	243.5	12030	0.020	1.7

Final substrate concentrations ranged from 0.1 nM to 75–500 μM of glucocorticoids, 0.5 nM–50 mM of NNK and 0.025–0.75 mM for oracin. In contrast to calculations by the Michaelis–Menten equation, statistically significant values were obtained for dehydrocorticosterone ($p < 0.001$), cortisone ($p < 0.02$), NNK ($p < 0.02$) and oracin reduction when the sigmoidal dose–response kinetic (according to the Hill-equation) was applied. V_{max}, K_m and Hill-values are derived from Maser et al. (2003) and Wsol et al. (2004). Oracin data calculated for [a](+)-DHO and [b](−)-DHO formation, respectively.

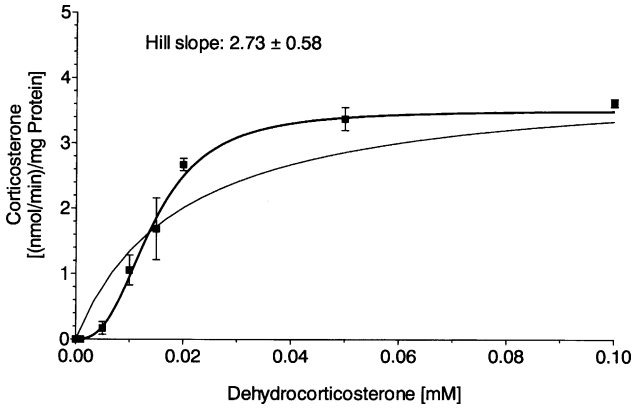

Fig. 3. Michaelis–Menten vs. sigmoidal dose–response kinetic of purified 11β-HSD1 for dehydrocorticosterone. Reduction of dehydrocorticosterone was determined over a wide substrate concentration range (0.1 nM–0.5 mM). In contrast to the Michaelis–Menten equation (calculating a hyperbola corresponding to a "one site binding" mode; thin curve), the sigmoidal dose–response calculation (a "two site binding" mode; bold curve) yielded a statistically significant ($p < 0.02$) variable slope (Hill-slope) of 2.73 ± 0.58, corresponding to a cooperative kinetic of 11β-HSD1. Kinetic values were calculated by using the GraphPad Prism computer software (Maser et al., 2003).

detoxification of NNK by providing the hydroxy moiety for glucuronidation via UTP-glucuronyltransferase (Maser et al., 1996, 2003; Maser, 1997, 1998). A problem in these considerations was the discrepancy between the high K_m values of 11β-HSD1 for NNK carbonyl reduction (low millimolar range, obtained by Michaelis–Menten calculation) and the low NNK levels observed in smokers (nanomolar concentrations). This discrepancy was also found for cytosolic NNK carbonyl reductases identified in a later study (Atalla et al., 2000). However, as observed with glucocorticoids, NNK carbonyl reduction by 11β-HSD1 showed cooperative kinetics (Table 1). The sigmoid dose–response kinetics of 11β-HSD1 for NNK may highlight the special importance of this enzyme in view of the relatively low levels of NNK observed in active and passive smokers.

Glycyrrhetinic acid, a constituent of licorice, is known as a classical inhibitor of 11β-HSD1 (Stewart et al., 1987). Importantly, we found that glycyrrhetinic acid inhibits 11β-HSD1 mediated NNK carbonyl reduction in nanomolar concentrations (Maser et al., 2003). Licorice, however, in addition to being a confectionery, serves as a major tobacco additive which is used in cigarette manufacturing in milligram amounts per cigarette as taste and flavour intensifier (Carmines, 2002). It is conceivable that licorice ingestion or licorice supplementation to tobacco prevents NNK detoxification by inhibiting NNK carbonyl reduction (Maser, 1997; Maser, 2004) (Fig. 4). This condition may advance lung cancer incidence, especially in smokers expressing low levels of 11β-HSD1.

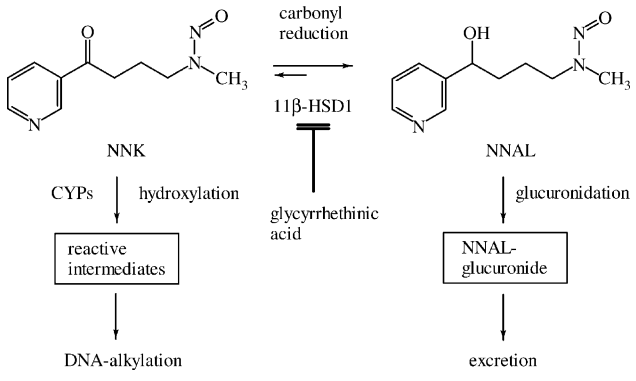

Fig. 4. Main metabolic pathways of NNK in humans. Simplified scheme of the metabolic fate of the tobacco-derived carcinogen NNK in humans (adopted from: Maser, 1997, 2004). NNK detoxification might be affected by glycyrrhetinic acid, the main constituent of licorice. Licorice, however, is added to cigarette tobacco in milligram per gram amounts as an additive to prevent mucosa irritation during smoking.

5.2. Expression of 11β-HSD1 in human lung

RT-PCR, Northern blot and immunohistochemical analyses have localized 11β-HSD1 to glucocorticoid target tissues such as liver, lung, testis, adipose tissue and others. Recently, we have examined the interindividual variability of 11β-HSD1 expression in human lung by RT-PCR (Soldan et al., 1999). Levels of 11β-HSD1 mRNA varied over an almost 20-fold range among different subjects (Fig. 5). These large interindividual differences in 11β-HSD1/NNK carbonyl reductase expression may have implications regarding the risk to lung cancer of smokers.

5.3. Role of 11β-HSD1 in the detoxification of the anti-cancer drug oracin

11β-HSD1 was also shown to catalyze the reduction of further important xenobiotic carbonyl compounds including the prospective anti-tumor drug oracin (Wsol et al., 2004). Oracin is a promising cytostatic drug, which is presently in phase II of clinical trials. As observed with glucocorticoid and NNK reduction oracin carbonyl reduction showed cooperative kinetics. Chiral studies on the stereospecificity of 11β-HSD1 revealed a ratio of 3:1 (*R*:*S* enantiomers) upon reduction of the pro-chiral carbonyl

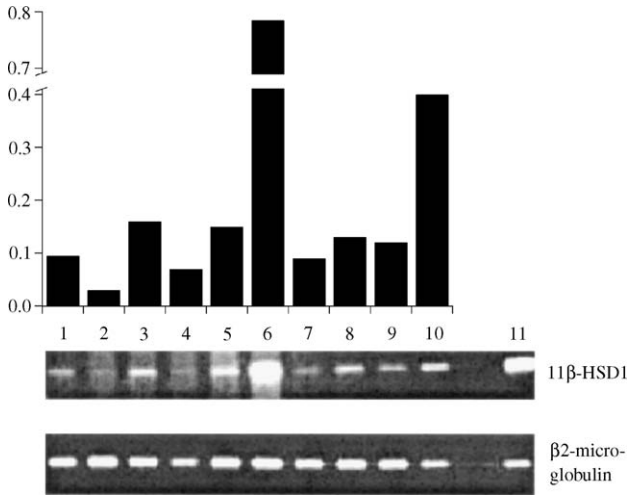

Fig. 5. Expression of 11β-HSD1 in human lung. RT-PCR has been performed in 10 different human lung samples (lanes 1–10) (Soldan et al., 1999). Compared to β2-microglobulin as internal standard great interindividual variations of up to 20-fold could be observed. Lane 11 represents a positive control and contains DNA from a lamda ZAP human liver cDNA library.

centre of oracin (Table 1). However, carbonyl reduction leads to the inactivation of oracin and is unwanted since it protects cancer cells from being killed by this chemotherapeutic. If 11β-HSD1 inhibition is beneficial in a treatment setting to enhance the cytostatic activity of oracin in vivo requires further study.

6. Conclusion

11β-HSD1 was shown to catalyze the reduction of important xenobiotic carbonyl compounds including the tobacco-specific nitrosamine NNK and the prospective anti-tumor drug oracin. As observed with glucocorticoid reduction, xenobiotic carbonyl reduction showed cooperative kinetics indicating that the enzyme may dynamically adapt to low as well as to high substrate concentrations. The fact that 11β-HSD1 is able to catalyze the metabolism of endogenous steroids as well as that of non-steroidal carbonyl compounds suggests interactions between the endocrine system and the detoxification of xenobiotics on the level of hydroxysteroid dehydrogenases.

It may be worthwhile mentioning that compared with oxidative cytochrome P450, carbonyl reducing enzymes which do also belong to the group of phase I drug-metabolizing enzymes have received considerably less attention. However, the advancement of carbonyl reductase molecular biology has allowed the identification of several pluripotent hydroxysteroid dehydrogenases that are additionally involved in xenobiotic carbonyl compound detoxification (Maser, 1995). These enzymes could be classified into either the short-chain dehydrogenase/reductase (SDR) or aldo–keto reductase (AKR) protein superfamilies. As the hydroxysteroid dehydrogenase/carbonyl reductase project continues to progress, it is anticipated that new members of these groups of enzymes, which play important roles in not only the metabolism of xenobiotics but also the biotransformation of a variety of endogenous steroids, are bound to emerge. The major challenge then will probably be to elucidate their enzymatic characteristics, physiological roles and substrate specificities.

In conclusion, our data expand insights into the multifunctional nature of 11β-HSD1 and emphasize the importance of this enzyme in drug metabolism, in addition to its endocrinological functions. As an example, low expression of 11β-HSD1 and/or inhibition by glycyrrhetinic acid (licorice) may explain why “only” some 20% of smokers develop lung cancer. It will be of interest in the future to correlate the expression of 11β-HSD1 in diseased and normal subjects in an effort to understand the protective role of the enzyme against tobacco-smoke related lung cancer.

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References

Agarwal, A.K., Tusie Luna, M.T., Monder, C., White, P.C., 1990. Expression of 11β-hydroxysteroid dehydrogenase using recombinant vaccinia virus. Mol. Endocrinol. 4, 1827–1832.

Atalla, A., Breyer-Pfaff, U., Maser, E., 2000. Purification and characterization of oxidoreductases-catalyzing carbonyl reduction of the tobacco-specific nitrosamine 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) in human liver cytosol. Xenobiotica 30, 755–769.

Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: cigarette design, testing approach, and review of results. Food Chem. Toxicol. 40, 77–91.

Diederich, S., Hanke, B., Burkhardt, P., Muller, M., Schoneshofer, M., Bahr, V., Oelkers, W., 1998. Metabolism of synthetic corticosteroids by 11β-hydroxysteroid-dehydrogenases in man. Steroids 63, 271–277.

Hecht, S.S., 1998. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem. Res. Toxicol 11, 559–603.

Maser, E., 1998. 11β-Hydroxysteroid dehydrogenase responsible for carbonyl reduction of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung microsomes. Cancer Res. 58, 2996–3003.

Maser, E., 1995. Xenobiotic carbonyl reduction and physiological steroid oxidoreduction—the pluripotency of several hydroxysteroid dehydrogenases. Biochem. Pharmacol. 49, 421–440.

Maser, E., 1997. Stress, hormonal changes, alcohol, food constituents and drugs: factors that advance the incidence of tobacco smoke-related cancer? Trends Pharmacol. Sci. 18, 270–275.

Maser, E., 2004. Significance of reductases in the detoxification of the tobacco-specific carcinogen NNK. Trends Pharmacol. Sci. 25, 235–237.

Maser, E., Friebertshäuser, J., Völker, B., 2003. Purification, characterization and NNK carbonyl reductase activities of 11β-hydroxysteroid dehydrogenase type 1 from human liver: enzyme cooperativity and significance in the detoxification of a tobacco-derived carcinogen. Chem. Biol. Interact. 143–144, 435–448.

Maser, E., Oppermann, U.C.T., 1997. Role of type-1 11β-hydroxysteroid dehydrogenase in detoxification processes. Eur. J. Biochem. 249, 356–369.

Maser, E., Richter, E., Friebertshauser, J., 1996. The identification of 11β-hydroxysteroid dehydrogenase as carbonyl reductase of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Eur. J. Biochem. 238, 484–489.

Maser, E., Volker, B., Friebertshauser, J., 2002. 11β-Hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase. Biochemistry 41, 2459–2465.

Moore, C.C., Mellon, S.H., Murai, J., Siiteri, P.K., Miller, W.L., 1993. Structure and function of the hepatic form of 11β-hydroxysteroid dehydrogenase in the squirrel monkey, an animal model of glucocorticoid resistance. Endocrinology 133, 368–375.

Pu, X., Yang, K., 2000. Guinea pig 11β-hydroxysteroid dehydrogenase type 1: primary structure and catalytic properties. Steroids 65, 148–156.

Soldan, M., Nagel, G., Losekam, M., Ernst, M., Maser, E., 1999. Interindividual variability in the expression and NNK carbonyl reductase activity of 11β-hydroxysteroid dehydrogenase 1 in human lung. Cancer Lett. 145, 49–56.

Stewart, P.M., Valentino, R., Wallace, A.M., Burt, D., Shackleton, C.H.L., Edwards, C.R.W., 1987. Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age. Lancet ii, 821–824.

Wsol, V., Szotakova, B., Skalova, L., Maser, E., 2004. The novel anticancer drug oracin: different stereospecificity and cooperativity for carbonyl reduction by purified human liver 11β-hydroxysteroid dehydrogenase type 1. Toxicology 197, 253–261.