Formation of Threohydrobupropion from Bupropion Is Dependent on 11β-Hydroxysteroid Dehydrogenase 1

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ABSTRACT

Bupropion is widely used for treatment of depression and as a smoking-cessation drug. Despite more than 20 years of therapeutic use, its metabolism is not fully understood. While CYP2B6 is known to form hydroxybupropion, the enzyme(s) generating erythro- and threohydrobupropion have long remained unclear. Previous experiments using microsomal preparations and the nonspecific inhibitor glycyrrhetinic acid suggested a role for 11β-hydroxysteroid dehydrogenase (11β-HSD1) in the formation of both erythro- and threohydrobupropion. 11β-HSD1 catalyzes the conversion of inactive glucocorticoids (cortisone, prednisone) to their active forms (cortisol, prednisolone). Moreover, it accepts several other substrates. Here, we used for the first time recombinant 11β-HSD1 to assess its role in the carbonyl reduction of bupropion. Furthermore, we applied human, rat, and mouse liver microsomes and a selective inhibitor to characterize species-specific differences and to estimate the relative contribution of 11β-HSD1 to bupropion metabolism. The results revealed 11β-HSD1 as the major enzyme responsible for threohydrobupropion formation. The reaction was stereoselective and no erythrohydrobupropion was formed. Human liver microsomes showed 10 and 80 times higher activity than rat and mouse liver microsomes, respectively. The formation of erythrohydrobupropion was not altered in experiments with microsomes from 11β-HSD1-deficient mice or upon incubation with 11β-HSD1 inhibitor, indicating the existence of another carbonyl reductase that generates erythrohydrobupropion. Molecular docking supported the experimental findings and suggested that 11β-HSD1 selectively converts R-bupropion to threohydrobupropion. Enzyme inhibition experiments suggested that exposure to bupropion is not likely to impair 11β-HSD1-dependent glucocorticoid activation but that pharmacological administration of cortisone or prednisone may inhibit 11β-HSD1-dependent bupropion metabolism.

Introduction

Bupropion ([(±)-1-(3-chlorophenyl)-2-[(1,1-dimethylethyl) amino]-1-propanone]; Wellbutrin; GlaxoSmithKline, Research Triangle Park, NC) has been used for the treatment of depression for more than 20 years (Holm and Spencer, 2000). It is also administered as a smoking-cessation drug (Zyban). Furthermore, bupropion has recently been proposed for the treatment of attention-deficit/hyperactivity disorders (Jafarinia et al., 2012). According to a recent review, approximately 40 million patients worldwide have been treated with bupropion (Fava et al., 2005). Despite its frequent use, the mechanisms of bupropion metabolism are not fully understood. The identification and characterization of the enzymes involved may help to optimize the therapeutic use of bupropion and to avoid potential drug-drug interactions.

Therapeutically, bupropion is used as a racemic mixture of R- and S-bupropion and acts as a dopamine and norepinephrine reuptake inhibitor. The first studies with bupropion in humans in the 1980s led to the identification of the three major metabolites: hydroxybupropion, erythrohydrobupropion, and threohydrobupropion (Schroeder, 1983; Laizure et al., 1985; Martin et al., 1990; Wang et al., 2010); however, the enzymes responsible for its metabolism remained unknown. A decade later, CYP2B6 was identified as the enzyme responsible for the formation of hydroxybupropion (Faucette et al., 2000; Hesse et al., 2000). Another ten years later, experiments with human and baboon placental and liver microsomes and the nonspecific 11β-hydroxysteroid dehydrogenase (11β-HSD) inhibitor 18β-glycyrrhetinic acid (GA) suggested that bupropion is metabolized by one of the 11β-HSDs to erythrohydrobupropion and threohydrobupropion (Wang et al., 2010, 2011; Molnari and Myers, 2012). Incubation with the nonspecific...
that 11β-HSD1 is responsible for conversion of the inactive 11-ketoglucocorticoids cortisone (humans) and 11-dehydrocorticosterone (rodents) to the active 11β-hydroxyglucocorticoids cortisol (humans) and corticosterone (rodents), whereas 11β-HSD2 catalyzes the reverse reaction (White et al., 1997). 11β-HSD2 plays a crucial role in protecting mineralocorticoid receptors from activation by glucocorticoids (Odermatt and Kratschmar, 2012). Although 11β-HSD2 is able to act as a reversible enzyme for some substrates, such as dexamethasone/11-ketodexamethasone, under in vitro conditions (Rebuffat et al., 2004), it functions exclusively as a dehydrogenase in vivo, and a role in the reduction of bupropion can be excluded. 11β-HSD1 is expressed in many metabolically active tissues, such as liver, adipose, and skeletal muscle (Atanasov and Odermatt, 2007). In addition to the reduction of cortisone, 11β-HSD1 essentially converts the prodrug prednisone to its active form prednisolone (Hult et al., 1998), thereby enabling activation of the glucocorticoid receptor and regulating glucocorticoid-receptor-dependent target genes. Due to the adverse metabolic effects of prolonged periods of exposure to excessive glucocorticoid levels and the observed metabolic disturbances in transgenic mice overexpressing 11β-HSD1 in adipose tissue (Masuzaki and Flier, 2003), there are considerable efforts to develop inhibitors for the treatment of metabolic syndrome, with ongoing phase II trials (Hughes et al., 2008; An et al., 2013; Anagnostis et al., 2013; Gatherecle et al., 2013; Venier et al., 2013). In addition, 11β-HSD1 inhibitors are currently under investigation for the treatment of several other diseases, including osteoporosis, glaucoma, age-associated impaired cognitive function, aging skin, and wound healing (Gathercole et al., 2013; Luo et al., 2013; Tiganescu et al., 2013).

Nevertheless, 11β-HSD1 is a multifunctional carboxyl reductase with broad substrate specificity (Odermatt and Nashef, 2010). It is able to reduce endogenous steroids such as 7-cholesterol (Hult et al., 2004; Schweizer et al., 2004), the secondary bile acid 7-oxocholeholic acid (Odermatt et al., 2011), 7-ketodehydroepiandrosterone (Nashef et al., 2007), and several xenobiotics, including triadimefon (Meyer et al., 2013), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK; nicotine-derived nitrosamine ketone) (Maser et al., 1998; Schweizer et al., 2004), the secondary bile acid 7-deoxycholic acid in the for-
using four parametric logistic curve fitting (GraphPad Prism; GraphPad Software, Inc., La Jolla, CA).

**Liquid Chromatography–Tandem Mass Spectrometry Measurements.** An Acquity ultra-performance liquid chromatography (UPLC) BEH C18 column (1.7-µm particle size, 130-Å pore diameter, 2.1-mm internal diameter × 150-mm column length, ID: Waters, Milford, MA) and an Agilent 1290 Infinity Series chromatograph (Agilent Technologies, Basel, Switzerland) were used for chromatographic separations.

The mobile phase consisted of solvent A (H2O/glacial acetic acid, 95:5 (v/v), containing 0.1% formic acid), and solvent B (H2O/glacial acetic acid, 5:95 (v/v), containing 0.1% formic acid), at a flow rate of 0.5 ml/minute. Bupropion, hydroxybupropion, threo-hydroxybupropion, and erythro-hydroxybupropion were separated using 15% solvent B for 6 minutes, followed by a linear gradient from 6 to 10 minutes to reach 100% solvent B, and then 100% solvent B for 3 minutes. The column was then re-equilibrated with 15% solvent B. Cortisone and cortisol were resolved as described earlier (Meyer et al., 2013).

The UPLC was interfaced to an Agilent 6490 triple quadrupole tandem mass spectrometer. The entire UPLC–tandem mass spectrometer system was controlled by MassHunter Workstation software, version B.01.05 (Agilent Technologies). The injection volume of each sample was 5 μl. The mass spectrometer was operated in electrospray ionization positive ionization mode, a source temperature of 350°C, a nebulizer pressure of 20 psi, and a capillary voltage of 4000 V.

The compounds were analyzed using multiple-reaction-monitoring and identified by comparing their retention time and mass-to-charge ratios (m/z) with those of authentic standards. The transitions, collision energy, and retention time were m/z 240.1/184.1, 19 V, and 4.9 minutes for bupropion; m/z 242/168, 20 V, and 5.4 minutes for threo-hydroxybupropion, m/z 242/168, 20 V, and 4.8 minutes for erythro-hydroxybupropion; m/z 256/238.1, 17 V, and 3.0 minutes for hydroxybupropion, and m/z 216/174, 16 V, and 5 minutes for the internal standard atrazine.

The UPLC–tandem mass spectrometer method was validated for accuracy, precision, sensitivity, recovery, and calibration range. Acceptable interday assay precision (± 6.2%) and accuracy (94.1–105.0%) were achieved over a linear range of 50–5000 nM for bupropion, hydroxybupropion, threo-hydroxybupropion, and erythro-hydroxybupropion. Recovery of bupropion, hydroxybupropion, threo-hydroxybupropion, and erythro-hydroxybupropion were 96, 80, 79, and 82%, respectively, in all extractions. For each experiment a new calibration curve was determined.

**Molecular Modeling.** The 2D structures of R- and S-bupropion were generated using ChemBioDraw Ultra 12.0 (1986-2010 CambridgeSoft; PerkinElmer, Waltham, MA). The 2D structures were converted into 3D structures using ChemBio3D Ultra 12.0 (1986-2010 CambridgeSoft). The docking studies were performed using GOLD (Cambridge Crystallographic Data Centre, Cambridge, UK) (Jones et al., 1997; Verdonk et al., 2003), which uses a genetic algorithm to produce low-energy binding solutions for small molecules in the ligand-binding pocket. The X-ray crystal structure of 11β-HSD1 was obtained from the Protein Data Bank (www.pdb.org) (Berman et al., 2000). Both stereoisomers of bupropion were docked into the ligand-binding site of 11β-HSD1 (PDB ID 2BEL, Chain A) (X. Wu et al., submitted manuscript). The binding site was defined as a 10-Å sphere, centered on the hydroxyl-oxygen of Ser170 (x: 3.84, y: 22.49, and z: 13.34). The protein side chains were handled as rigid and the ligand conformations as flexible during the docking run. The program was set to define the atom types of the ligands and the protein automatically. GoldScore was selected as a scoring function. The program was allowed to terminate the docking run in cases where three best-ranked solutions were within an root-mean-square-deviation of 1.0 Å from each other. Using these settings, the program successfully reproduced the binding mode of the cocystalized ligand carbamazepine, thus validating the docking settings.

**Results**

**Carbonyl Reduction of Bupropion by Human, Rat, and Mouse Liver Microsomes.** Earlier studies using the nonspecific 11β-HSD inhibitor GA and microsomes prepared from human placenta (Wang et al., 2010) and liver (Molnari and Myers, 2012) or from baboon liver (Wang et al., 2011) suggested a role for 11β-HSD enzymes in the metabolism of bupropion. To test our assumption that 11β-HSD1 catalyzes the carbonyl reduction of bupropion, we first measured the metabolism of bupropion in human liver microsomes that were incubated in the presence of G6P. In intact liver microsomes the lumen of the endoplasmic reticulum (ER) is protected by the microsomal membrane; these microsomes contain an endogenous NADPH-regenerating system consisting of hexose-6-phosphate dehydrogenase (Meyer et al., 2013). Therefore, 11β-HSD1 reductase activity can be measured by simultaneous incubation of microsomes with G6P and the substrate. Upon incubation with G6P and bupropion, human liver microsomes efficiently formed threo-hydroxybupropion and to a lesser extent (4–5-fold) erythro-hydroxybupropion (Fig. 1). Surprisingly, the selective 11β-HSD1 inhibitor T0504 completely blocked the formation of threo-hydroxybupropion but had no effect on the formation of erythro-hydroxybupropion.

To assess possible species-specific differences, we compared the activities of human, rat, and mouse liver microsomes. The rat and mouse liver microsomes were 10- and 80-fold less active than human liver microsomes in generating threo-hydroxybupropion. It is important to note that under the same conditions rat liver microsomes showed a 2-fold higher capacity to reduce the substrate cortisol than human or mouse liver microsomes, which had comparable activities (Meyer et al., 2013). Rat liver microsomes formed equal amounts of threo-hydroxybupropion and erythro-hydroxybupropion, whereas mouse liver microsomes formed about 2-fold more erythro-hydroxybupropion than threo-hydroxybupropion. As with the human liver microsomes, the 11β-HSD1 inhibitor T0504 selectively blocked threo-hydroxybupropion, suggesting that 11β-HSD1 stereoselectively reduces bupropion to threo-hydroxybupropion. To further support a role for 11β-HSD1 in bupropion metabolism, we used liver microsomes from liver-specific 11β-HSD1 knockout mice. Threo-hydroxybupropion formation was completely abolished, while erythro-hydroxybupropion formation was unaffected and comparable to that in wild-type mice, suggesting that another enzyme is responsible for the formation of erythro-hydroxybupropion.

![Fig. 1. Carbonyl reduction of bupropion by human, rat, and mouse liver microsomes.](image-url)
Impact of Cofactor on Bupropion Metabolism. As reported recently, the preparation of rodent microsomes used in this study yields intact vesicles with approximately 90% showing an orientation such that the luminal compartment is protected by the vesicular membrane and the cytoplasmic side is facing the solution. Thus, these microsomal preparations show about 90% latent activities of luminal enzymes. The commercially available human liver microsomes showed about 75% latency (Meyer et al., 2013). Nevertheless, incubation of human liver microsomes with G6P yielded approximately 8-fold higher amounts of threohydrobupropion than erythrohydrobupropion, but only minor amounts of hydroxybupropion (Fig. 2). As expected, incubation of microsomes with NADPH mainly led to the cytochrome P450-dependent formation of hydroxybupropion. The formation of threohydrobupropion is probably due to the microsomal fraction with reverse orientation, because its formation could be completely blocked by the 11β-HSD1 inhibitor T0504. Similar observations were made with mouse and rat liver microsomes, and even higher differences between NADPH- and G6P-dependent formation of hydroxybupropion versus erythro and threohydrobupropion, respectively, were measured (data not shown).

To roughly estimate the relative activities of cytochrome P450-dependent hydroxylation and 11β-HSD1-dependent carbonyl reduction in vitro, human liver microsomes were incubated in the presence of both NADPH and G6P (Fig. 3). Threohydrobupropion was the major product formed, followed by hydroxybupropion and erythrohydrobupropion, suggesting that 11β-HSD1-dependent threohydrobupropion formation is a major route of bupropion metabolism in humans.

Carbonyl Reduction of Bupropion by Recombinant Human 11β-HSD1 Measured in Cell Lysates. The lysates of HEK-293 cells transiently transfected with human 11β-HSD1 efficiently converted bupropion to threohydrobupropion (Fig. 4). Importantly, no other metabolites were detected, and lysates of untransfected HEK-293 cells did not metabolize bupropion. These incubations were performed in the presence of NADPH, because the cells were lysed by sonication to obtain multilamellar vesicles and vesicles with mixed orientation, therefore allowing direct access of NADPH to 11β-HSD1. An apparent $K_m$ of 2.1 ± 0.9 μM and $V_{max}$ of 0.22 ± 0.03 nmol/mg per hour for the carbonyl reduction of bupropion was obtained for human 11β-HSD1, suggesting that bupropion is less efficiently reduced by 11β-HSD1 than cortisone ($K_m$ of 0.34 ± 0.04 μM and $V_{max}$ of 1.88 ± 0.23 nmol/mg per hour) (Frick et al., 2004). Furthermore, we assessed whether 11β-HSD1 catalyzes the reverse reaction by incubating cell lysates with threohydrobupropion and NADP$. No bupropion could be detected under the conditions applied, indicating that 11β-HSD1 exclusively catalyzes the reduction reaction under physiologic conditions (data not shown).

Inhibition of 11β-HSD1-Dependent Cortisone Reduction by Bupropion and Vice Versa. To test whether the substrates influence each other, we first assessed the effect of bupropion on glucocorticoid activation. The reduction of cortisone was inhibited with an IC$_{50}$ value of 165 ± 51 μM (Fig. 5). Next, we tested the impact of cortisone and the widely used synthetic glucocorticoid prednisone on the carbonyl reduction of bupropion. The conversion of bupropion to threohydrobupropion was inhibited by cortisone and prednisone with IC$_{50}$ of 193 ± 40 nM (Fig. 6A) and 2.9 ± 0.3 μM, respectively (Fig. 6B).

Binding Mode Prediction of Bupropion to 11β-HSD1 by Molecular Docking. Both enantiomers of bupropion geometrically fit the binding site of 11β-HSD1, and both are predicted to bind next to...
the catalytic triad Ser170-Tyr183-Lys187 and the cofactor NADPH. However, the stereochecmistry of these two enantiomers allows only one of them, R-bupropion, to be metabolized by 11β-HSD1. Since the hydrogens in the reduction reaction are transferred to the substrate via the cofactor and Tyr183 (Oppermann et al., 1997; Kavanagh et al., 2008), it is essential that the carbonyl-oxygen of bupropion is located next to these residues. This is the case for R-bupropion (Fig. 7A): the carbonyl oxygen points toward the hydroxyl of Tyr183 with a distance of 1.92 Å, and the cofactor is at 2.46 Å distance from the carbonyl-carbon. In contrast, S-bupropion is located in the same place, but because of the different stereochemistry, the tert-butyl-group points toward the cofactor, thus pushing the carbonyl group further away from the hydroxyl of Tyr183 (3.32 Å) and the cofactor (4.11 Å), respectively (Fig. 7B). Thus, the S-bupropion carbonyl group is more distant from the catalytic H-donors and has a nonfavorable interaction angle with the Tyr183 hydroxyl group. These docking results support our biologic findings that 11β-HSD1 exclusively forms threo-hydrobupropion. Erythrohydrobupropion is not formed because of steric hindrance coming from the stereochemistry of S-bupropion.

Discussion

Based on earlier studies using microsomes from human and baboon liver and placenta, together with the nonspecific inhibitor GA, it was suggested that 11β-HSD enzymes are involved in the formation of both erythrohydrobupropion and threo-hydrobupropion (Wang et al., 2010, 2011; Molnari and Myers, 2012). However, since GA might inhibit other enzymes, the relative contribution of 11β-HSD enzymes remained unclear. In the present study, we used liver microsomes and the highly selective 11β-HSD1 inhibitor T0504, also known as Merck-544 (Arampatzis et al., 2005; Hermanowski-Vosatka et al., 2005), as well as recombinant 11β-HSD1 to characterize the carbonyl reduction of bupropion.

The comparison of human, rat, and mouse liver microsomes revealed clearly that human liver microsomes are more active than either rat or mouse liver microsomes in catalyzing the carbonyl reduction of bupropion, and threo-hydrobupropion was the preferred metabolite formed (Fig. 1). These findings provide an explanation for the observations by Welch et al., who found low levels of these metabolites in plasma of mice and rats (Welch et al., 1987). Furthermore, these authors reported that hydroxybupropion was a major urinary metabolite in human, mouse, and dog, whereas rats predominantly excreted side-chain cleavage products of bupropion such as m-chlorobenzoic acid. It was proposed that the distinct metabolism of bupropion might account for the species-specific pharmacological response of bupropion. Thus, our findings further support earlier studies indicating that rodents are not adequate models for the prediction of bupropion metabolism in humans.

The specific 11β-HSD1 inhibitor completely abolished the formation of threo-hydrobupropion from the racemic mixture of bupropion by liver microsomes from all three species, without affecting the formation of erythrohydrobupropion. Importantly, microsomes from liver-specific knockout mice were unable to generate threo-hydrobupropion, but the formation of erythrohydrobupropion was comparable to that by wild-type mouse liver microsomes. These results indicate that 11β-HSD1 is the major if not the only enzyme responsible for the formation of threo-hydrobupropion and emphasize the existence of another carbonyl reductase responsible for the formation of erythrohydrobupropion. The fact that erythrohydrobupropion is generated upon incubation of microsomes with G6P indicates that the unknown enzyme is localized within the ER, as is 11β-HSD1, and is dependent on hexose-6-phosphate dehydrogenase activity. We speculate that the unknown NADPH-dependent oxoreductase enzyme belongs to the short-chain dehydrogenase/reductase family. Currently, 11β-HSD1 is the only NADPH-dependent enzyme of this family that has been demonstrated to face the ER lumen; however, the function and intracellular localization of 30–40% of the members of this family (currently 72 members are known in the human genome) still remain unknown.

Using the recombinant enzyme, and under the conditions applied, we observed that human 11β-HSD1 exclusively catalyzes the carbonyl reduction of bupropion to threo-hydrobupropion. Analysis

![Fig. 5. Inhibition of 11β-HSD1-dependent reduction of cortisone by bupropion. Lysates of HEK-293 cells transiently transfected with human 11β-HSD1 were incubated with 1 μM cortisone, 1 mM NADPH, and different concentrations of cortisone (A) or prednisone (B) for 60 minutes at 37°C. Data were normalized to vehicle control (0.05% dimethyl sulfoxide) and represent mean ± S.D. from three independent experiments.](https://dmd.aspetjournals.org)
of the binding of bupropion and its metabolites to 11β-HSD1 by molecular modeling indicates that R-bupropion adopts a favorable binding position in the substrate pocket of 11β-HSD1, allowing electron transfer from the cofactor to form threohydrobupropion. In contrast, steric hindrance prevents optimal binding of S-bupropion and erythrohydrobupropion, suggesting that electron transfer is unlikely to occur. Unfortunately, pure S- and R-bupropion are currently not commercially available and will need to be tested in a future study to verify the prediction by molecular modeling.

In a study on exercise performance and neuroendocrine responses to exercise, the effect of bupropion on plasma adrenocorticotropic hormone and cortisol was measured in eight healthy well-trained male cyclists (Piacentini et al., 2004). While bupropion did not affect performance, it did slightly enhance ACTH and cortisol at the end of exercise, suggesting a central noradrenergic effect on the hormonal response to exercise. In another study, the response to a single dose of sustained-release bupropion on nocturnal urinary free cortisol was determined in 20 patients with unipolar major depressive disorder (Rao et al., 2005). Interestingly, bupropion significantly increased nocturnal urinary free cortisol in individuals not responding to the antidepressant effect, whereas no such change could be detected in responders. The nocturnal urinary free cortisol positively correlated with the severity of depression symptoms at the end of the treatment. The authors suggested that a differential sensitivity of the noradrenergic and/or dopaminergic system might be responsible for the observed effects. These findings suggest an effect of bupropion on hypothalamic-pituitary-adrenal activity. To start to understand whether administration of bupropion might interfere with intracellular 11β-HSD1-dependent glucocorticoid activation, we determined IC₅₀ for cortisol reduction. Given the rapid metabolism of bupropion in vivo (Welch et al., 1987) and the high IC₅₀ of 165 ± 51 μM of bupropion to inhibit cortisone reduction, it is unlikely that exposure to bupropion will significantly inhibit the 11β-HSD1-dependent conversion of endogenous cortisone to cortisol.

On the other hand, cortisone efficiently inhibited the carbonyl reduction of bupropion. The low IC₅₀ value of cortisone to inhibit
bupropion reduction suggests that pharmacological use as well as elevated endogenous cortisone levels during stress may abolish the concomitant carbonyl reduction of bupropion. The pharmacological use of prednisone is also likely to inhibit the 11β-HSD1-dependent carbonyl reduction of bupropion. An oral administration of a dose of 100-mg prednisone results in Cmax values of about 600 nM (Czock et al., 2005). Intrahepatic drug concentrations after first pass through the liver can be several-fold higher than circulating concentrations, suggesting that prednisone concentrations equal to or higher than the IC50 of 2.9 ± 0.3 μM obtained in the in vitro assay to inhibit bupropion carbonyl reduction may be reached.

Bupropion and its metabolites show different potency regarding the inhibition of biogenic amine uptake; they also differ in half-life and area under the curve (Laizure et al., 1985; Golden et al., 1988; Martin et al., 1990; Hsyu et al., 1997; Horst and Preskorn, 1998; Jefferson et al., 2005). It has been described earlier that hydroxybupropion, the metabolite generated by CYP2B6, has the highest potency (Schoeder, 1983; Martin et al., 1990). Pharmacological administration of cortisone and prednisone, high endogenous cortisone during stress, or the use of 11β-HSD1 inhibitors (currently in development to treat metabolic syndrome and other diseases (An et al., 2013; Anagnostis et al., 2013; Gathercole et al., 2013; Luo et al., 2013; Tiganescu et al., 2013; Venier et al., 2013) are likely to result in higher hydroxybupropion levels, which will necessitate a readjustment of the therapeutic dose of conventional antidepressants. Subjects receiving hormone replacement therapy, which leads to inhibition of CYP2B6, had diminished hydroxybupropion levels and increased erythro- and threo-hydrobupropion levels (Palovaara et al., 2003).

It has been shown that the glucuronides of erythro- and hydroxybupropion account for 13% of the urinary excretion of bupropion in man after a single 200-mg dose of bupropion (Welch et al., 1987). The localization of both 11β-HSD1 and the UDP-glucuronosyltransferase enzymes on the luminal side of the ER membrane facilitates glucuronidation of the newly formed threo- and threo-hydrobupropion. Similarly, our results indicate a luminal orientation of the unknown enzyme responsible for the formation of hydroxybupropion, which would facilitate subsequent glucuronidation. An impaired carbonyl reduction of bupropion by the unknown enzyme and by 11β-HSD1 is expected to result in delayed excretion, which may enhance the pharmacological effects of bupropion and hydroxybupropion.

In conclusion, our results indicate that 11β-HSD1 exclusively catalyzes the carbonyl reduction of R-bupropion to threo-hydrobupropion and suggest that another ER luminal enzyme is responsible for the formation of erythro-hydrobupropion (Fig. 8). 11β-HSD1-dependent carbonyl reduction of bupropion is about 10 and 80 times more efficient with human enzymes than with rat or mouse enzymes, while cortisone reduction is less than 2-fold different between these three species. Whereas bupropion is unlikely to impair 11β-HSD1-dependent glucocorticoid activation, the metabolism of bupropion is expected to be inhibited by high endogenous cortisone or pharmacological cortisone or prednisone, and dose adjustments of bupropion might be necessary to achieve optimal therapeutic effects. Further studies are needed to identify the ER luminal enzyme responsible for erythro-hydrobupropion formation and to examine the consequences of 11β-HSD1 inhibition on bupropion metabolism in humans.

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