DMD Fast Forward. Published on June 26, 2013 as doi:10.1124/dmd.113.052936

Formation of threohydrobupropion from bupropion is dependent on 11β-hydroxysteroid dehydrogenase 1

Arne Meyer, Anna Vuorinen, Agnieszka E. Zielinska, Petra Strajhar, Gareth G. Lavery, 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, Basel, Switzerland (A.M., P.S., A.O.)

Institute of Pharmacy/Pharmaceutical Chemistry and Center for Molecular Biosciences Innsbruck – CMBI, University of Innsbruck, Innsbruck, Austria (A.V., D.S.)

Centre for Endocrinology Diabetes and Metabolism (CEDAM), Institute of Biomedical Research, Medical School Building, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom (A.E.Z., G.G.L.)

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title:

Carbonyl reduction of bupropion by 11β-HSD1

C**orrespondence to**: Dr. Alex Odermatt, Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland. Phone: +41 61 267 1530, Fax: +41 61 267 1515, E-mail: alex.odermatt@unibas.ch

The Number of

Text Pages: 28

Tables: 0

Figures: 8

References: 53

Words in *Abstract*: 249

Words in *Introduction*: 733

Words in *Discussion*: 1278

Abbreviations:

11β-HSD, 11β-hydroxysteroid dehydrogenase; DMEM, Dulbecco's modified eagles medium; ER, endoplasmic reticulum; G6P, glucose-6-phosphate; GA, 18β-glycyrrhetinic acid; GR, glucocorticoid receptor; H6PDH, hexose-6-phosphate dehydrogenase; HEK, human embryonic kidney; HLM, human liver microsomes; LKO, liver-specific *HSD11B1* knockout mice; MLM, mouse liver microsomes; MR, mineralocorticoid receptor; MRM, multiple-reaction monitoring; RLM, rat liver microsomes.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Bupropion is widely used for treatment of depressions and as smoking cessation drug. Despite of 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 remained unclear. Previous experiments using microsomal preparations and the nonspecific inhibitor glycyrrhetinic acid (GA) suggested a role for 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) in the formation of both erythro- and threohydrobupropion. 11β-HSD1 catalyzes the conversion of inactive (cortisone, prednisone) to active glucocorticoids (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.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Bupropion ([(±)-1-(3-chlorophenyl)-2-[(1,1-dimethylethyl) amino]-1-propanone], Wellbutrin®) has been used for the treatment of depressions 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 of 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 the metabolism remained unknown. A decade later, cytochrome P450 2B6 (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; Wang et al., 2011; Molnari and Myers, 2012). Incubations with the nonspecific inhibitor GA yielded lower amounts of both threohydrobupropion and erythrohydrobupropion, suggesting the involvement of 11β-HSD1 in the carbonyl reduction of bupropion.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Two distinct 11β-HSD enzymes are known; 11β-HSD1 is responsible for the 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 (MR) 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 pro-drug prednisone to its active form prednisolone (Hult et al., 1998), thereby enabling activation of the glucocorticoid receptor (GR) and regulating GR-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 (An et al., 2013; Anagnostis et al., 2013; Gathercole et al., 2013; Venier et al., 2013). Besides, 11β-HSD1 inhibitors are currently investigated 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 multi-functional carbonyl reductase with broad substrate specificity (Odermatt and Nashev, 2010). It is able to reduce endogenous sterols such as 7-ketocholesterol (Hult et al., 2004; Schweizer et al., 2004), the secondary bile acid 7-oxolithocholic acid

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

(Odermatt et al., 2011), 7-ketodehydroepiandrosterone (Nashev et al., 2007) and several xenobiotics, including triadimefon (Meyer et al., 2013), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Maser et al., 2003), oracin (Wsol et al., 2003), metyrapone (Maser and Bannenberg, 1994) and ketoprofen (Hult et al., 2001).

The evidence from earlier studies, using microsomes and the nonspecific inhibitor GA, suggested a role for 11β-HSD1 in the formation of the two metabolites erythrohydrobupropion and threohydrobupropion. Since it still remained unclear whether indeed 11β-HSD1 is responsible for the generation of these two metabolites, and whether it plays a major or minor role, we used hepatic microsomes, a selective 11β-HSD1 inhibitor, and recombinant enzyme to assess the role of 11β-HSD1 in bupropion metabolism. Furthermore, we investigated species-specific differences in the carbonyl reduction of bupropion by human, rat and mouse liver microsomes. The contribution of 11β-HSD1 was further assessed using microsomes from liver-specific 11β-HSD1 knockout mice. Finally, the putative binding of bupropion to 11β-HSD1 was investigated by molecular modeling, suggesting that 11β-HSD1 selectively generates threohydrobupropion from *R*-bupropion.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Chemicals and reagents

Microsomes from a liver of a 77 year old male Caucasian were purchased from Celsis In Vitro Inc (Baltimore, MD). Human embryonic kidney (HEK-293) cells from ATCC (No CRL-1573) were purchased from LGC Standards S.a.r.l. (Molsheim Cedex, France). Cell culture medium was purchased from Invitrogen (Carlsbad, CA), tricyclo[3.3.1.13,7]dec-1-yl-6,7,8,9-tetrahydro-5H-1,2,4-triazolo[4,3-a]azepine (T0504) from Enamine (Kiev, Ukraine), and steroids from Steraloids (Newport, RI). The metabolites hydroxybupropion, erythrohydrobupropion and threohydrobupropion were purchased from Toronto Research Chemicals Inc. (North York, Canada), and bupropion and all other chemicals from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). The solvents were of analytical and high performance liquid chromatography grade and reagents of the highest grade available.

Cell culture and transfection

HEK-293 cells were grown at 37 °C in Dulbecco's modified Eagle medium (DMEM, containing 4.5 g/L glucose, 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/mL streptomycin, 1 × MEM non-essential amino acids and 10 mM HEPES buffer, pH 7.4). For the experiments with recombinant 11β-HSD1, HEK-293 cells were transiently transfected by the calcium phosphate transfection method as described earlier (Meyer et al., 2013) with plasmids for human, rat or mouse 11β-HSD1 (Arampatzis et al., 2005). Cells were harvested 48 h post-transfection, centrifuged at 900 × g for 4 min, and cell pellets were immediately shock frozen and stored at -80°C until further use. Protein concentration was determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA).

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Preparation of liver microsomes

Microsomes were prepared as described earlier (Meyer et al., 2013). Livers were taken from adult male Sprague Dawley rats, C57BL/6J mice and liver-specific knock-out mice (LKO) generated by crossing albumin-Cre transgenic mice on a C57BL/6J background with floxed homozygous *HSD11B1* mice on a mixed C57BL/6J/129SvJ background (Lavery et al., 2012). Liver tissue was homogenized, and microsomes were obtained after differential centrifugation as described (Meyer et al., 2013). Microsomes were finally resuspended in a buffer containing 0.15 M potassium chloride, 0.25 M sucrose, and 10 mM Tris-maleate, pH 7.0. Aliquots were stored at -80°C until further use. The microsomal protein concentration was measured using the Pierce BCA protein assay kit. The quality of the microsomal preparations was analyzed using the cytochrome C reductase assay kit (Sigma-Aldrich Chemie GmbH) and by assessing the latent activity of the 11β-HSD1-dependent oxoreduction of cortisone in the presence of glucose-6-phosphate (G6P).

Enzyme activity measurements using liver microsomes

The oxoreduction of cortisone by liver microsomes was measured as reported earlier (Meyer et al., 2013). The metabolism of bupropion was determined at 37 °C (1 h incubation) in a final reaction volume of 22 µL of TS2 buffer (100 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM $MgCl_2$, 250 mM sucrose, 20 mM Tris–HCl, pH 7.4) containing 1 µM of bupropion and either human liver microsomes (final concentration (f.c.) of 0.4 mg/mL) or rat, mouse or LKO mouse liver microsomes (all at a f.c. of 1 mg/mL), supplemented with either 1 mM G6P or 1 mM NADPH in the presence or absence of 20 µM of the selective 11β-HSD1 inhibitor T0504. Reactions were stopped by adding 200 µL 0.3 M zinc sulfate in a 1:1 (v/v) mixture of water and

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

methanol. Atrazine was added as an internal standard at an f.c. of 50 nM, followed by vortexing for 10 s and centrifugation for 10 min at 12,000 × g on a table top centrifuge. Samples were further purified by an ethyl acetate extraction. Supernatants (180 µL) were added to 600 µL ethyl acetate and incubated for 10 min on a thermomixer at 700 rpm. Following centrifugation for 10 min at 12,000 × g, supernatants (550 µL) were evaporated to dryness, reconstituted in 100 µL methanol and stored at -20°C until analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS)(see below).

Enzyme activity measurements using lysates of transfected HEK-293 cells

Frozen pellets of HEK-293 cells transiently expressing human, rat or mouse 11β-HSD1 were resuspended in TS2 buffer and sonicated. Lysates were then incubated for 1 h at 37 °C in the presence of 1 mM NADPH and different concentrations of bupropion (8 µM, 4 µM, 2 µM, 1 µM, 500 nM, 250 nM and 125 nM) in a final volume of 22 µL to estimate apparent K_M and apparent V_{max} values. Substrate conversion was kept below 25% in all experiments. Reactions were stopped and processed as described above.

For measuring the reductase activity of 11β-HSD1, cell lysates were incubated in the presence of 1 µM cortisone or 1 µM bupropion as substrate and various concentrations of either bupropion or cortisone and prednisone as the respective inhibitor. IC_{50} values were calculated by non-linear regression using four parametric logistic curve fitting (GraphPad Prism software).

Liquid chromatography-tandem mass spectrometry measurements

An Acquity 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 (H_2O/acetonitrile, 95:5 (v/v), containing 0.1% formic acid, and solvent B (H_2O/acetonitrile, 5:95 (v/v), containing 0.1% formic acid, at a flow rate of 0.5 mL/min. Bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion were separated using 15% solvent B for 6 min, followed by a linear gradient from 6 to 10 min to reach 100% solvent B, and then 100% solvent B for 3 min. 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 quadropole tandem mass spectrometer (MS/MS). The entire UPLC-MS/MS system was controlled by Mass Hunter workstation software (version B.01.05). The injection volume of each sample was 5 µL. The mass spectrometer was operated in electrospray ionization (ESI) 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 (MRM) and identified by comparing their retention time and mass to charge ratio (*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 min for bupropion; *m/z* 242/168, 20 V and 5.4 min for threohydrobupropion, *m/z* 242/168, 20 V and 4.8 min for erythrohydrobupropion; *m/z* 256/238.1, 17 V and 3.0 min for hydroxybupropion and *m/z* 216/174, 16 V and 5 min for the internal standard atrazine.

The UPLC-MS/MS method was validated for accuracy, precision, sensitivity, recovery, and calibration range. Acceptable inter-day assay precision (≤ 6.2%) and accuracy (94.1 – 105.0%) were achieved over a linear range of 50 to 5000 nM for bupropion, hydroxybupropion, threohydrobupropion and erythrohydrobupropion. Recovery of bupropion, hydroxybupropion,

threohydrobupropion and erythrohydrobupropion 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). The 2D-structures were converted into 3D-structures using ChemBio3D Ultra 12.0 (1986-2010 CambridgeSoft). The docking studies were performed using GOLD (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 code 2BEL, Chain A (Wu)). 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 RMSD of 1.0 Å from each other. Using these settings, the program successfully reproduced the binding mode of the cocrystallized ligand carbenoxolone, thus validating the docking settings.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

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. Intact liver microsomes, where the ER lumen is protected by the microsomal membrane, contain an endogenous NADPH regenerating system consisting of hexose-6-phosphate dehydrogenase (H6PDH) (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 threohydrobupropion and to a lesser extent (4-5 fold) erythrohydrobupropion (Fig. 1). Surprisingly, the selective 11β-HSD1 inhibitor T0504 completely blocked the formation of threohydrobupropion but had no effect on the formation of erythrohydrobupropion.

To assess possible species-specific differences, we compared the activities of human, rat and mouse liver microsomes. The rat and mouse liver microsomes showed 10- and 80-fold lower activities than human liver microsomes to generate threohydrobupropion. It is important to note that under the same conditions rat liver microsomes showed a two-fold higher activity to reduce the substrate cortisone than human and mouse liver microsomes, which had comparable activities (Meyer et al., 2013). Rat liver microsomes formed equal amounts of threohydrobupropion and erythrohydrobupropion and mouse liver microsomes about 2-fold more erythrohydrobupropion than threohydrobupropion. As with the human liver microsomes, the 11β-HSD1 inhibitor T0504 selectively blocked threohydrobupropion, suggesting that 11β-HSD1 stereo-selectively reduces

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

bupropion to threohydrobupropion. To further support a role for 11β-HSD1 in bupropion metabolism, we used liver microsomes from liver-specific 11β-HSD1 knockout mice (LKO). Threohydrobupropion formation was completely abolished, while erythrohydrobupropion formation was unaffected and comparable to that in wild-type mice, suggesting that another enzyme is responsible for the formation of erythrohydrobupropion.

Impact of cofactor on bupropion metabolism

As reported recently, the preparation of rodent microsomes applied yields intact vesicles with approximately 90% showing an orientation where 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,

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

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 in order to obtain multi-lamellar 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/h 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/h, (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 physiological 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

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

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 to 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 stereochemistry 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 towards 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 towards 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 non-favorable interaction angle with the Tyr183 hydroxyl group. These docking results support our biological findings that exclusively threohydrobupropion is formed by 11β-HSD1. Erythrohydrobupropion is not formed because of steric hindrance coming from the stereochemistry of *S*-bupropion.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

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 threohydrobupropion (Wang et al., 2010; Wang et al., 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 highest activity of human liver microsomes to catalyze the carbonyl reduction of bupropion, and threohydrobupropion 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 may 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 threohydrobupropion from the racemic mixture of bupropion by liver microsomes from all three species, without affecting the formation of erythrohydrobupropion. Importantly, microsomes from liver-specific knock-out mice were unable to generate threohydrobupropion, 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 threohydrobupropion 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 H6PDH 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 threohydrobupropion. Analysis 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 the 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 the exercise performance and neuroendocrine response to exercise, the effect of bupropion on plasma ACTH 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

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

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 may 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 IC_{50} for cortisone reduction. Regarding the rapid metabolism of bupropion *in vivo* (Welch et al., 1987) and the high IC_{50} 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 IC_{50} 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 also is likely to inhibit the 11β-HSD1-dependent carbonyl reduction of bupropion. An oral administration of a dose of 100 mg prednisone results in C_{max} 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 or higher than the IC_{50} of 2.9 ± 0.3 µM obtained in the *in vitro* assay to inhibit bupropion carbonyl reduction may be reached.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Bupropion and its metabolites show different potency regarding the inhibition of biogenic amine uptake, different half-life and AUC (Laizure et al., 1985; Golden Rn, 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 (Schroeder, 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 need a readjustment of the therapeutic dose of bupropion. Subjects receiving hormone replacement therapy, which leads to inhibition of CYP2B6 had diminished hydroxybupropion levels and increased erythro- and threohydrobupropion levels (Palovaara et al., 2003).

It has been shown that the glucuronides of erythro- and threohydrobupropion 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-glucuronosyl transferase enzymes on the luminal side of the ER membrane facilitates glucuronidation of the newly formed threohydrobupropion. Similarly, our results suggest a luminal orientation of the unknown enzyme responsible for the formation of erythrohydrobupropion, which would facilitate subsequent glucuronidation. An impaired carbonyl reduction of bupropion by the unknown enzyme and by 11β-HSD1 is expected to result in a delayed excretion, which may enhance the pharmacological effect of bupropion and hydroxybupropion.

In conclusion, our results indicate that 11β-HSD1 exclusively catalyzes the carbonyl reduction of *R*-bupropion to threohydrobupropion and suggest that another ER luminal enzyme is responsible for the formation of erythrohydrobupropion (Fig. 8). 11β-HSD1-dependent carbonyl reduction of

bupropion is about 10 and 80 times more efficient with the human compared to the rat and mouse enzymes, whereby cortisone reduction is less than two-fold different between these three species. Whereas bupropion unlikely impairs 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 erythrohydrobupropion formation and to examine the consequences of 11β-HSD1 inhibition on bupropion metabolism in humans.

Acknowledgements

We thank Thierry Da Cunha for technical assistance with UPLC-MS/MS.

Authorship contributions:

Participated in research design: Meyer, Vuorinen, Schuster, Lavery, Odermatt

Conducted experiments: Meyer, Vuorinen, Zielinska, Strajhar

Contributed new reagents or analytic tools: Schuster, Lavery, Odermatt

Performed data analysis: Meyer, Vuorinen, Schuster, Odermatt

Wrote or contributed to the writing of the manuscript: Meyer, Vuorinen, Lavery, Schuster, Odermatt

References

An G, Liu W, Katz DA, Marek G, Awni W, and Dutta S (2013) Effect of ketoconazole on the pharmacokinetics of the 11β-hydroxysteroid dehydrogenase type 1 inhibitor ABT-384 and its two active metabolites in healthy volunteers: Population analysis of data from a drug-drug interaction study. *Drug Metab Disp* 41:1035-1045.

Anagnostis P, Katsiki N, Adamidou F, Athyros VG, Karagiannis A, Kita M, and Mikhailidis DP (2013) 11β-Hydroxysteroid dehydrogenase type 1 inhibitors: novel agents for the treatment of metabolic syndrome and obesity-related disorders? *Metabolism* 62:21-33.

Arampatzis S, Kadereit B, Schuster D, Balazs Z, Schweizer RA, Frey FJ, Langer T, and Odermatt A (2005) Comparative enzymology of 11β-hydroxysteroid dehydrogenase type 1 from six species. *J Mol Endocrinol* 35:89-101.

Atanasov AG and Odermatt A (2007) Readjusting the glucocorticoid balance: an opportunity for modulators of 11β-hydroxysteroid dehydrogenase type 1 activity? *Endocr Metab Immune Disord Drug Targets* 7:125-140.

Berman HM, Westbrook J, Feng Z, Gililand G, Bhat TN, Weissig H, Shindyalov IN, and Bourne PE (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235-242.

Czock D, Keller F, Rasche FM, and Haussler U (2005) Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet* 44:61-98.

Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM, and Lindley CM (2000) Validation of Bupropion Hydroxylation as a Selective Marker of Human Cytochrome P450 2B6 Catalytic Activity. *Drug Metab Dispos* 28:1222-1230.

Fava M, Rush AJ, Thase ME, Clayton A, Stahl SM, Pradko JF, and Johnston JA (2005) 15 years of clinical experience with bupropion HCl: from bupropion to bupropion SR to bupropion XL. *Prim Care Companion J Clin Psychiatry* 7:106-113.

Frick C, Atanasov AG, Arnold P, Ozols J, and Odermatt A (2004) Appropriate function of 11β-hydroxysteroid dehydrogenase type 1 in the endoplasmic reticulum lumen is dependent on its N-terminal region sharing similar topological determinants with 50-kDa esterase. *J Biol Chem* 279:31131-31138.

Gathercole LL, Lavery GG, Morgan SA, Cooper MS, Sinclair AJ, Tomlinson JW, and Stewart PM (2013) 11β-Hydroxysteroid Dehydrogenase 1: Translational and Therapeutic Aspects. *Endocrine Reviews* doi: 10.1210/er.2012-1050.

Golden Rn, DeVane C, Laizure S, Rudorfer MV, Sherer MA, Potter WZ (1988) Bupropion in depression: Ii. the role of metabolites in clinical outcome. *Arch Gen Psychiat* 45:145-149.

Hermanowski-Vosatka A, Balkovec JM, Cheng K, Chen HY, Hernandez M, Koo GC, Le Grand CB, Li Z, Metzger JM, Mundt SS, Noonan H, Nunes CN, Olson SH, Pikounis B, Ren N, Robertson N, Schaeffer JM, Shah K, Springer MS, Strack AM, Strowski M, Wu K, Wu T, Xiao J, Zhang BB, Wright SD, and Thieringer R (2005) 11β-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *J Exp Med* 202:517-527.

Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI, and Greenblatt DJ (2000) CYP2B6 Mediates the In Vitro Hydroxylation of Bupropion: Potential Drug Interactions with Other Antidepressants. *Drug Metab Dispos* 28:1176-1183.

Holm KJ and Spencer CM (2000) Bupropion: A Review of its Use in the Management of Smoking Cessation. *Drugs* 59:1007-1024.

Horst WD and Preskorn SH (1998) Mechanisms of action and clinical characteristics of three atypical antidepressants: venlafaxine, nefazodone, bupropion. *J Affect Disord* 51:237-254.

Hsyu P-H, Singh A, Giargiari TD, Dunn JA, Ascher JA, and Johnston JA (1997) Pharmacokinetics of Bupropion and its Metabolites in Cigarette Smokers versus Nonsmokers. *J Clin Pharmacol* 37:737-743.

Hughes KA, Webster SP, and Walker BR (2008) 11β-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) inhibitors in type 2 diabetes mellitus and obesity. *Expert Opin Investig Drugs* 17:481-496.

Hult M, Elleby B, Shafqat N, Svensson S, Rane A, Jornvall H, Abrahmsen L, and Oppermann U (2004) Human and rodent type 1 11β-hydroxysteroid dehydrogenases are 7beta-hydroxycholesterol dehydrogenases involved in oxysterol metabolism. *Cell Mol Life Sci* 61:992-999.

Hult M, Jornvall H, and Oppermann UC (1998) Selective inhibition of human type 1 11β-hydroxysteroid dehydrogenase by synthetic steroids and xenobiotics. *FEBS Lett* 441:25-28.

Hult M, Nobel CS, Abrahmsen L, Nicoll-Griffith DA, Jornvall H, and Oppermann UC (2001) Novel enzymological profiles of human 11β-hydroxysteroid dehydrogenase type 1. *Chem Biol Interact* 130-132:805-814.

Jafarinia M, Mohammadi M-R, Modabbernia A, Ashrafi M, Khajavi D, Tabrizi M, Yadegari N, and Akhondzadeh S (2012) Bupropion versus methylphenidate in the treatment of children with attention-deficit/hyperactivity disorder: randomized double-blind study. *Hum Psychopharm Clin* 27:411-418.

Jefferson JW, Pradko JF, and Muir KT (2005) Bupropion for major depressive disorder: Pharmacokinetic and formulation considerations. *Clin Therapeut* 27:1685-1695.

Jones G, Willett P, Glen RC, Leach AR, and Taylor R (1997) Development and validation of a genetic algorithm for flexible docking. *J Mol Biol* 267:727-748.

Kavanagh KL, Jornvall H, Persson B, and Oppermann U (2008) Medium- and short-chain dehydrogenase/reductase gene and protein families : the SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. *Cell Mol Life Sci* 65:3895-3906.

Laizure SC, DeVane CL, Stewart JT, Dommisse CS, and Lai AA (1985) Pharmacokinetics of bupropion and its major basic metabolites in normal subjects after a single dose. *Clin Pharmacol Ther* 38:586-589.

Lavery GG, Zielinska AE, Gathercole LL, Hughes B, Semjonous N, Guest P, Saqib K, Sherlock M, Reynolds G, Morgan SA, Tomlinson JW, Walker EA, Rabbitt EH, and Stewart PM (2012) Lack of significant metabolic abnormalities in mice with liver-specific disruption of 11β-hydroxysteroid dehydrogenase type 1. *Endocrinology* 153:3236-3248.

Luo MJ, Thieringer R, Springer MS, Wright SD, Hermanowski-Vosatka A, Plump A, Balkovec JM, Cheng K, Ding GJ, Kawka DW, Koo GC, Grand CB, Luo Q, Maletic MM, Malkowitz L, Shah K, Singer I, Waddell ST, Wu KK, Yuan J, Zhu J, Stepaniants S, Yang X, Lum PY, and Wang IM (2013) 11β-HSD1 inhibition reduces atherosclerosis in mice by altering proinflammatory gene expression in the vasculature. *Physiol Genomics* 45:47-57.

Martin P, Massol J, Colin JN, Lacomblez L, and Puech AJ (1990) Antidepressant profile of bupropion and three metabolites in mice. *Pharmacopsychiatry* 23:187-194.

Maser E and Bannenberg G (1994) 11β-hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds. *Biochem Pharmacol* 47:1805-1812.

Maser E, Friebertshauser J, and Volker 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.

Masuzaki H and Flier JS (2003) Tissue-specific glucocorticoid reactivating enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) - a promising drug target for the treatment of metabolic syndrome. *Curr Drug Targets Immune Endocr Metabol Disord* 3:255-262.

Meyer A, Vuorinen A, Zielinska AE, Da Cunha T, Strajhar P, Lavery GG, Schuster D, and Odermatt A (2013) Carbonyl reduction of triadimefon by human and rodent 11β-hydroxysteroid dehydrogenase 1. *Biochem Pharmacol* 85:1370-1378.

Molnari JC and Myers AL (2012) Carbonyl reduction of bupropion in human liver. *Xenobiotica* 42:550-561.

Nashev LG, Chandsawangbhuwana C, Balazs Z, Atanasov AG, Dick B, Frey FJ, Baker ME, and Odermatt A (2007) Hexose-6-phosphate dehydrogenase modulates 11β-hydroxysteroid dehydrogenase type 1-dependent metabolism of 7-keto- and 7β-hydroxy-neurosteroids. *PLoS One* 2:e561.

Odermatt A, Da Cunha T, Penno CA, Chandsawangbhuwana C, Reichert C, Wolf A, Dong M, and Baker ME (2011) Hepatic reduction of the secondary bile acid 7-oxolithocholic acid is mediated by 11β-hydroxysteroid dehydrogenase 1. *Biochem J* 436:621-629.

Odermatt A and Kratschmar DV (2012) Tissue-specific modulation of mineralocorticoid receptor function by 11β-hydroxysteroid dehydrogenases: An overview. *Mol Cell Endocrinol* 350:168-186.

Odermatt A and Nashev LG (2010) The glucocorticoid-activating enzyme 11β-hydroxysteroid dehydrogenase type 1 has broad substrate specificity: Physiological and toxicological considerations. *J Steroid Biochem Mol Biol* 119:1-13.

Oppermann UC, Filling C, Berndt KD, Persson B, Benach J, Ladenstein R, and Jornvall H (1997) Active site directed mutagenesis of 3β-/17β-hydroxysteroid dehydrogenase establishes differential effects on short-chain dehydrogenase/reductase reactions. *Biochemistry* 36:34-40.

Palovaara S, Pelkonen O, Uusitalo J, Lundgren S, and Laine K (2003) Inhibition of cytochrome P450 2B6 activity by hormone replacement therapy and oral contraceptive as measured by bupropion hydroxylation. *Clin Pharmacol Ther* 74:326-333.

Piacentini MF, Meeusen R, Buyse L, De Schutter G, and De Meirleir K (2004) Hormonal responses during prolonged exercise are influenced by a selective DA/NA reuptake inhibitor. *Br J Sports Med* 38:129-133.

Rao U, Ott GE, Lin KM, Gertsik L, and Poland RE (2005) Effect of bupropion on nocturnal urinary free cortisol and its association with antidepressant response. *J Psychiatr Res* 39:183-190.

Rebuffat AG, Tam S, Nawrocki AR, Baker ME, Frey BM, Frey FJ, and Odermatt A (2004) The 11-ketosteroid 11-ketodexamethasone is a glucocorticoid receptor agonist. *Mol Cell Endocrinol* 214:27-37.

Schroeder DH (1983) Metabolism and kinetics of bupropion. *J Clin Psychiatry* 44:79-81.

Schweizer RA, Zurcher M, Balazs Z, Dick B, and Odermatt A (2004) Rapid hepatic metabolism of 7-ketocholesterol by 11β-hydroxysteroid dehydrogenase type 1: species-specific differences between the rat, human, and hamster enzyme. *J Biol Chem* 279:18415-18424.

Tiganescu A, Tahrani AA, Morgan SA, Otranto M, Desmouliere A, Abrahams L, Hassan-Smith Z, Walker EA, Rabbitt EH, Cooper MS, Amrein K, Lavery GG, and Stewart PM (2013) 11beta-hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest* doi:10.1172/JCI64162.

Venier O, Pascal C, Braun A, Namane C, Mougenot P, Crespin O, Pacquet F, Mougenot C, Monseau C, Onofri B, Dadji-Faïhun R, Leger C, Ben-Hassine M, Van-Pham T, Ragot J-L, Philippo C, Farjot G, Noah L, Maniani K, Boutarfa A, Nicolaï E, Guillot E, Pruniaux M-P, Güssregen S, Engel C, Coutant A-L, de Miguel B, and Castro A (2013) Discovery of SAR184841, a potent and long-lasting inhibitor of 11β-hydroxysteroid dehydrogenase type 1, active in a physiopathological animal model of T2D. *Bioorg Med Chem Lett* 23:2414-2421.

Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, and Taylor RD (2003) Improved protein-ligand docking using GOLD. *Proteins* 52:609-623.

Wang X, Abdelrahman DR, Fokina VM, Hankins GD, Ahmed MS, and Nanovskaya TN (2011) Metabolism of bupropion by baboon hepatic and placental microsomes. *Biochem Pharmacol* 82:295-303.

Wang X, Abdelrahman DR, Zharikova OL, Patrikeeva SL, Hankins GDV, Ahmed MS, and Nanovskaya TN (2010) Bupropion metabolism by human placenta. *Biochem Pharmacol* 79:1684-1690.

Welch RM, Lai AA, and Schroeder DH (1987) Pharmacological significance of the species differences in bupropion metabolism. *Xenobiotica* 17:287-298.

White PC, Mune T, and Agarwal AK (1997) 11β-Hydroxysteroid Dehydrogenase and the Syndrome of Apparent Mineralocorticoid Excess. *Endocr Rev* 18:135-156.

Wsol V, Szotakova B, Skalova L, and Maser E (2003) Stereochemical aspects of carbonyl reduction of the original anticancer drug oracin by mouse liver microsomes and purified 11β-hydroxysteroid dehydrogenase type 1. *Chem Biol Interact* 143-144:459-468.

Wu X, et al. The High Resolution Structures of Human, Murine and Guinea Pig 11β-Hydroxysteroid Dehydrogenase Type 1 Reveal Critical Differences in Active Site Architecture. *DOI:102210/pdb2bel/pdb.*

Footnotes

This work was supported by the Swiss National Science Foundation [Grant PDFMP3-127330] to Alex Odermatt and a BBSRC David Philips fellowship [Grant BB/G023468/1] to Gareth Lavery. Alex Odermatt has a Chair for Molecular and Systems Toxicology by the Novartis Research Foundation. Anna Vuorinen is supported by a PhD grant from the Austrian Academy of Sciences (ÖAW) and thanks the University of Innsbruck, Young Talents Grants (Nachwuchsförderung) for financial support. Daniela Schuster is financed by an Erika Cremer Habilitation Program from the University of Innsbruck.

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legends

Fig. 1. Carbonyl reduction of bupropion by human, rat and mouse liver microsomes. Human liver microsomes (HLM, final concentration (f.c.) 0.4 mg/mL), rat liver microsomes (RLM, f.c. 1 mg/mL), mouse liver microsomes (MLM, f.c. 1 mg/mL) and microsomes from livers of liver-specific 11β-HSD1-deficient mice (LKO, f.c. 1 mg/mL) were incubated for 1 h at 37°C with 1 µM bupropion and 1 mM glucose-6-phosphate (G6P), in the absence or presence of 20 µM of the 11β-HSD1 inhibitor T0504. Data (mean ± SD) were obtained from at least three independent experiments using pooled microsomes. *** $p < 0.001$, multiple measures ANOVA found significant species differences in bupropion reduction, post hoc analysis by Tukey test was used for multiple comparison.

Fig. 2. Impact of cofactor on the metabolism of bupropion by human liver microsomes. Human liver microsomes (f.c. 0.4 mg/mL) were incubated for 1 h at 37°C in the presence of 1 µM bupropion and either 1 mM NADPH or 1 mM glucose-6-phosphate (G6P). Data represent mean ± SD from at least three independent experiments using pooled microsomes. ns = not significant, * $p < 0.05$, *** $p < 0.001$, multiple measures ANOVA found significant differences in the groups, post hoc analysis by Tukey test was used for multiple comparison.

Fig. 3. Bupropion and its major metabolites after incubation of human liver microsomes with NADPH and G6P. Human liver microsomes (f.c. 0.2 mg/mL) were incubated for 1 h at 37°C in the presence of 1 µM bupropion, 1 mM NADPH and 1 mM G6P. Data represent mean ± SD from at least three independent experiments with pooled microsomes.

Fig. 4. Concentration-dependent reduction of bupropion to threohydrobupropion. HEK-293 cells transiently transfected with plasmid for human 11β-HSD1 were sonicated to obtain mixed vesicles, followed by incubation for 1 h at 37°C in the presence of 1 mM NADPH and different concentrations of bupropion as given in Materials and Methods. Apparent K_M (2.1 µM ± 0.9 µM)

DMD Fast Forward. Published on June 26, 2013 as DOI: 10.1124/dmd.113.052936
This article has not been copyedited and formatted. The final version may differ from this version.

and apparent V_{max} (0.22 ± 0.03 nmol/mg/h) values were calculated. Data represent mean ± SD from at least three independent experiments.

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 bupropion for 15 min at 37°C. Data were normalized to vehicle control (0.05% DMSO) and represent mean ± SD from three independent experiments.

Fig. 6. Inhibition of 11β-HSD1-dependent threohydrobupropion reduction by cortisone and prednisone. Lysates of HEK-293 cells transiently transfected with human 11β-HSD1 were incubated with 1 μM bupropion, 1 mM NADPH and different concentrations of cortisone (A) or prednisone (B) for 60 min at 37°C. Data were normalized to activity of vehicle control (0.05% DMSO) and represent mean ± SD from three independent experiments.

Fig. 7. Proposed binding modes of *R*-bupropion and *S*-bupropion in the ligand binding pocket of human 11β-HSD1. *R*-bupropion (A) is colored in green and *S*-bupropion (B) in blue. The catalytic triad and the cofactor are colored in grey. The distances between the substrate and the protein are given in Å.

Fig. 8. Schematic model of bupropion metabolism.

Figure 1

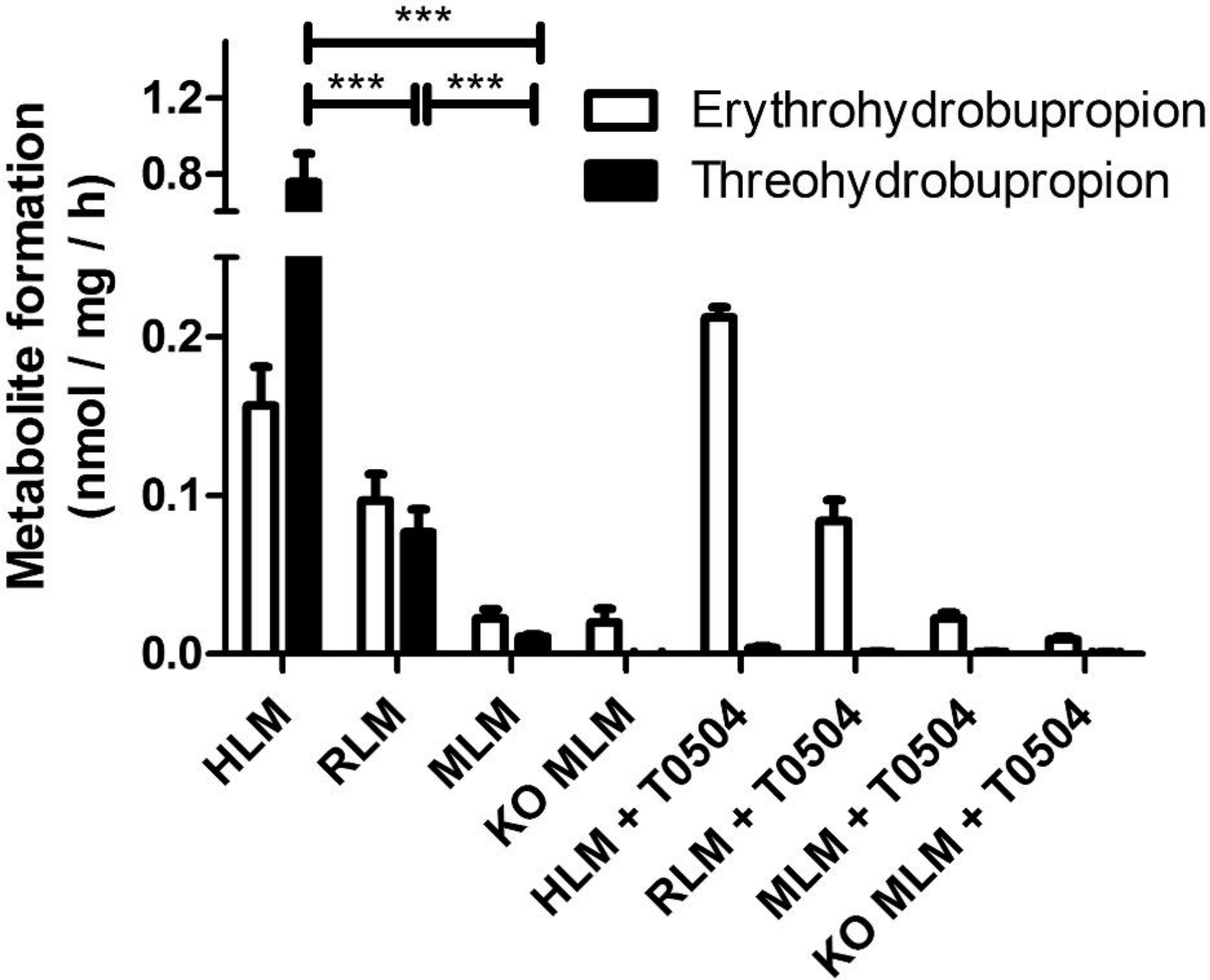

***
***
***
1.2
0.8
0.2
0.1
0.0
Metabolite formation
(nmol / mg / h)
Erythrohydrobupropion
Threohydrobupropion
HLM
RLM
MLM
KO MLM
HLM + T0504
RLM + T0504
MLM + T0504
KO MLM + T0504

Figure 2

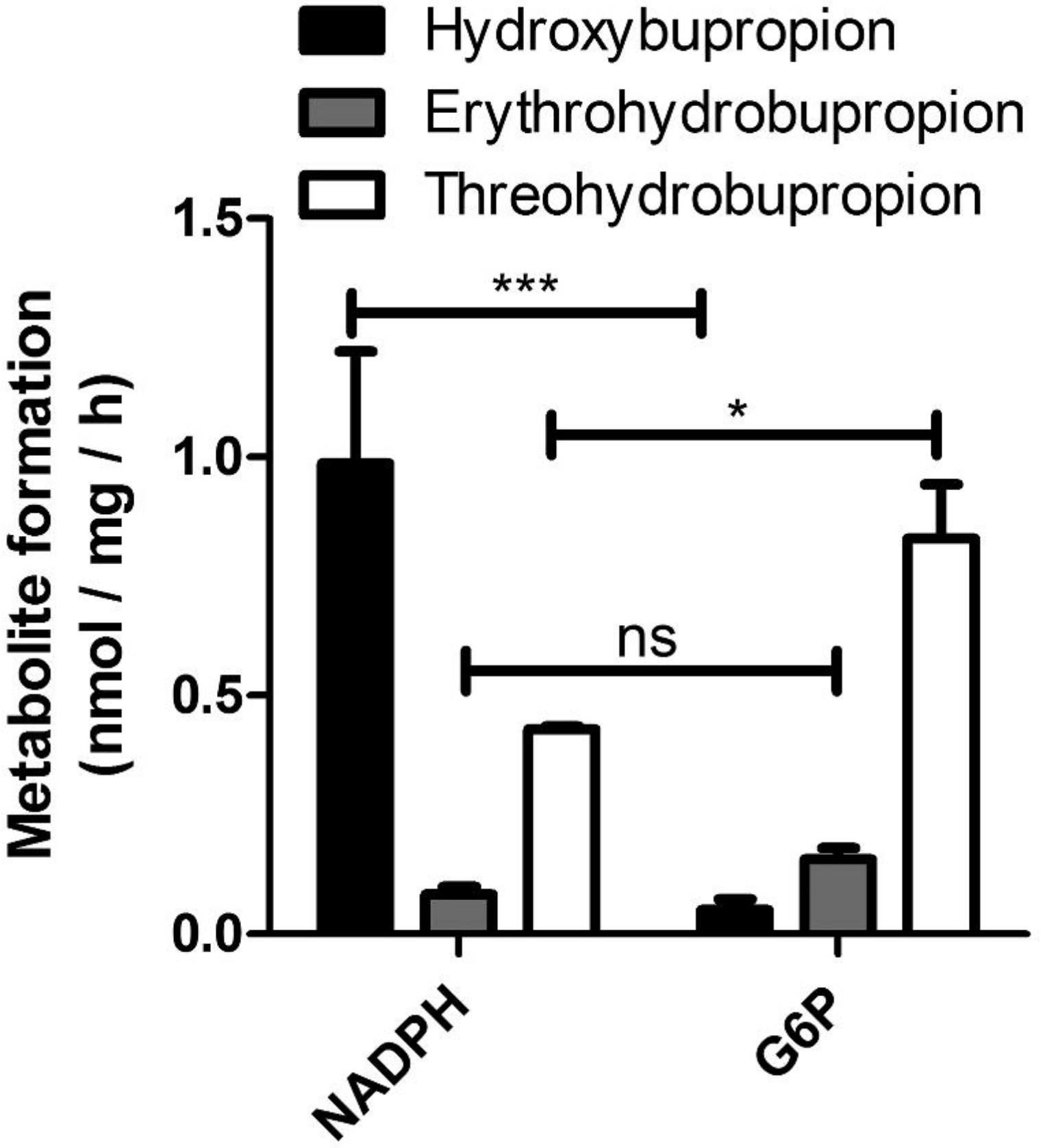

Hydroxybupropion
Erythrohydrobupropion
Threohydrobupropion
Metabolite formation
(nmol / mg / h)
1.5
1.0
0.5
0.0
***
*
ns
NADPH
G6P

Figure 3

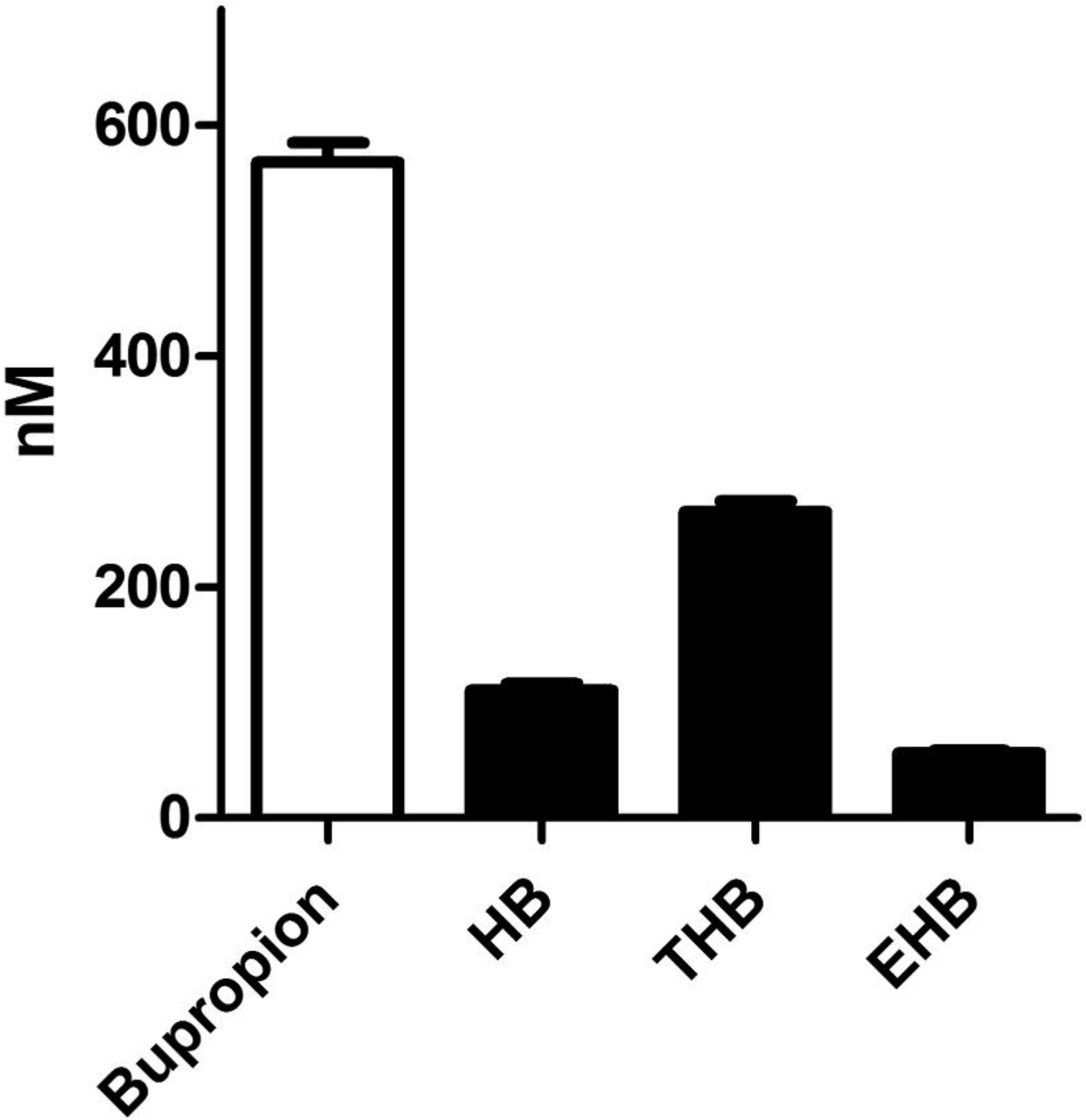


Figure 4

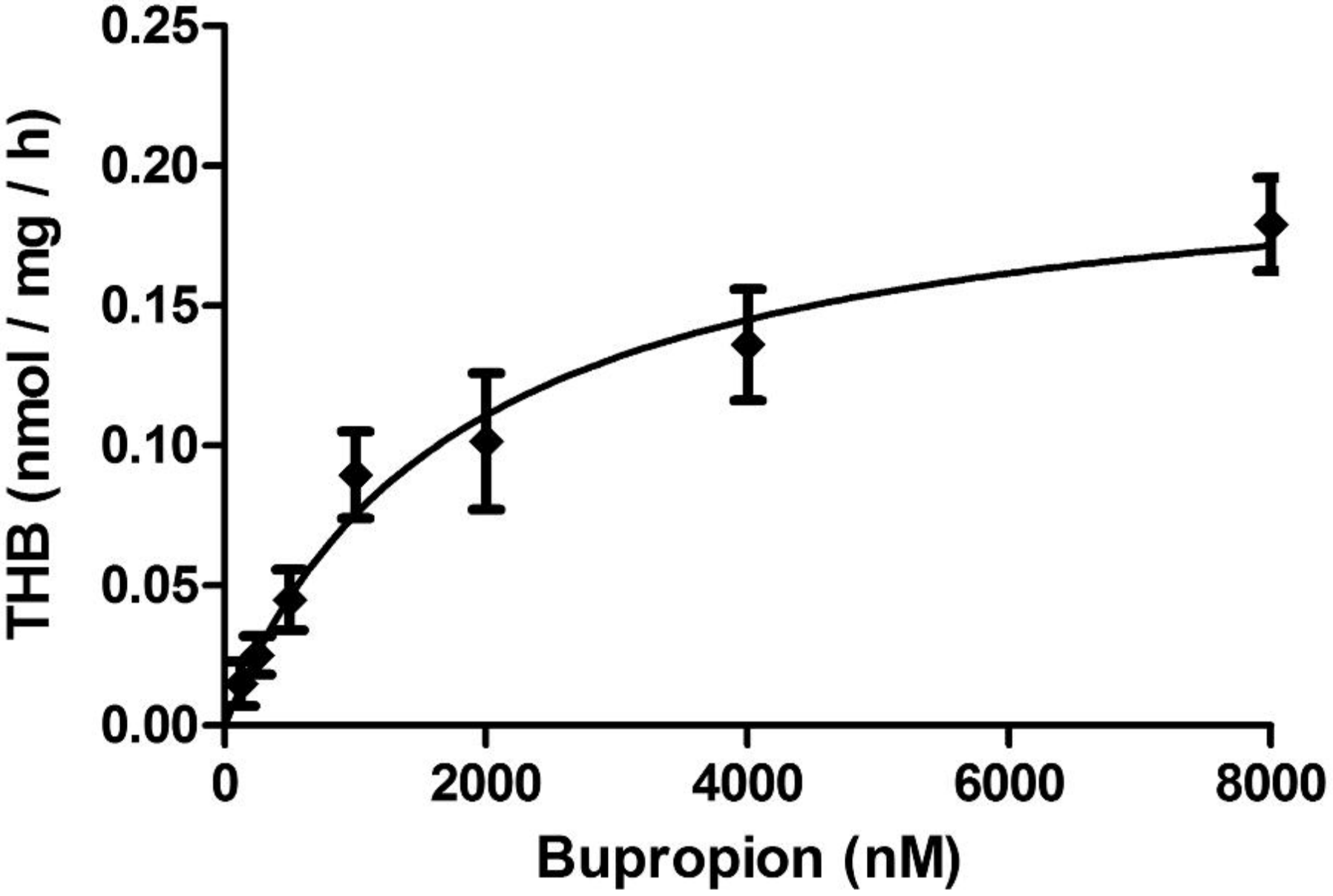

0.25
0.20
0.15
0.10
0.05
0.00
THB (nmol / mg / h)
0
2000
4000
6000
8000
Bupropion (nM)

Figure 5

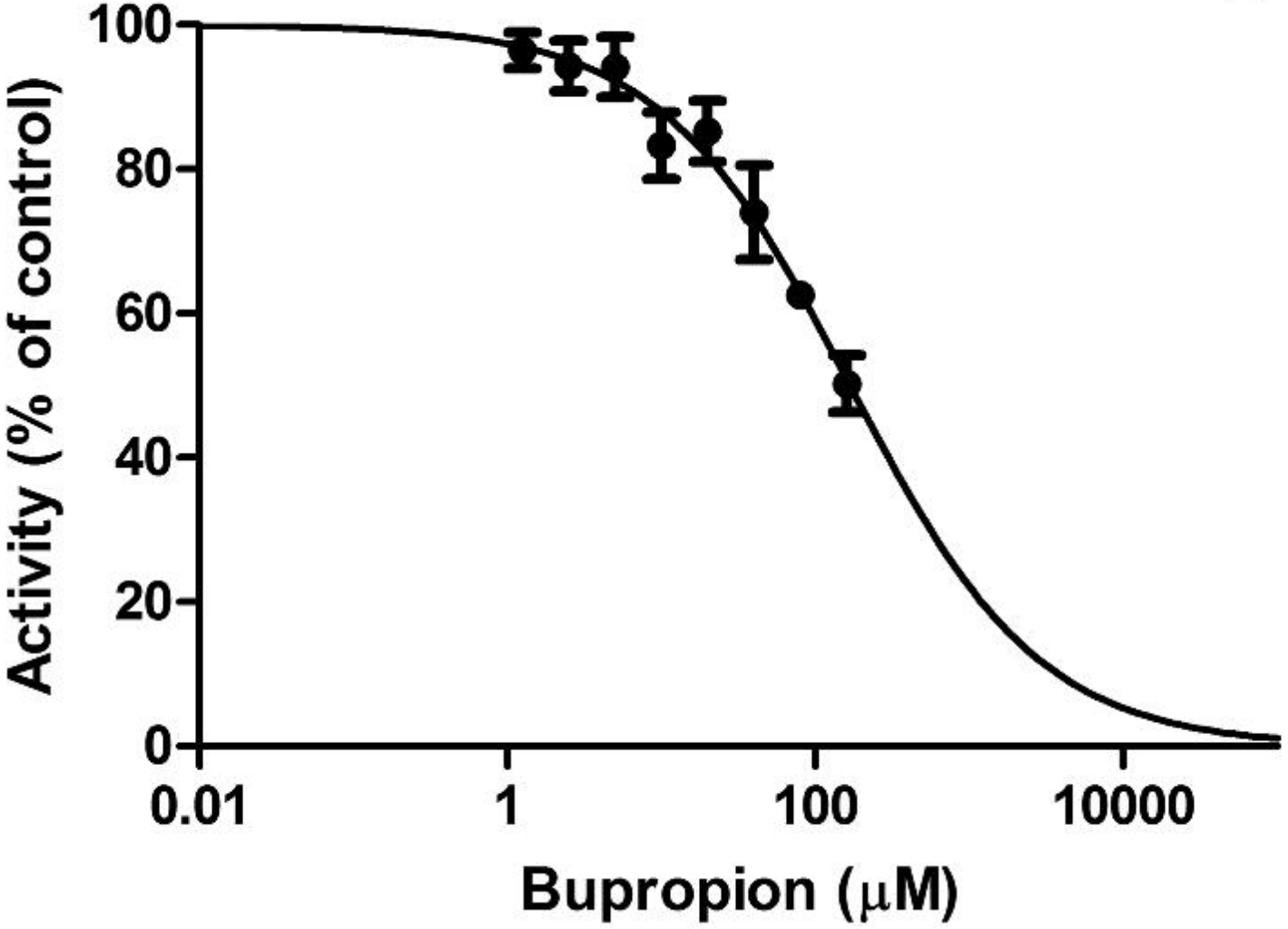
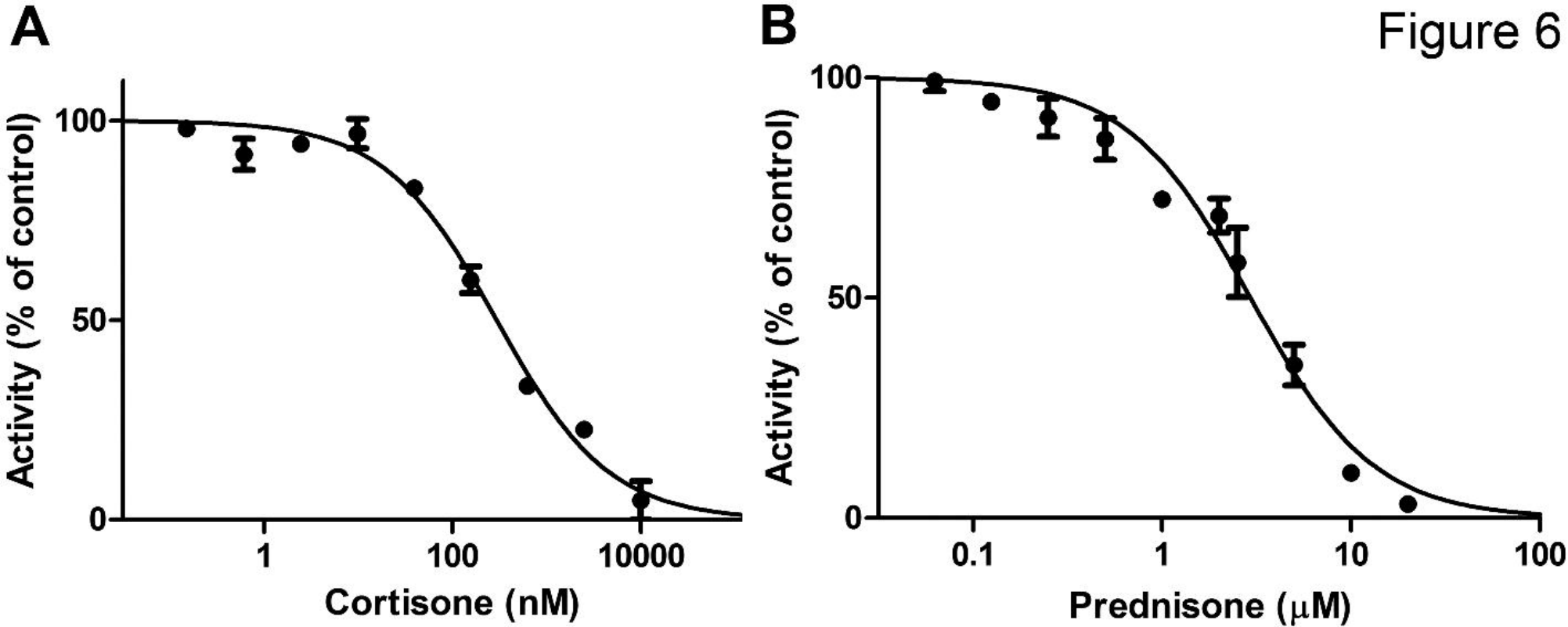

100
80
60
40
20
0
Activity (% of control)
0.01
1
100
10000
Bupropion (μM)

Figure 6


A
Activity (% of control)
100
50
0
1
100
10000
Cortisone (nM)
B
Activity (% of control)
100
50
0
0.1
1
10
100
Prednisone (μM)

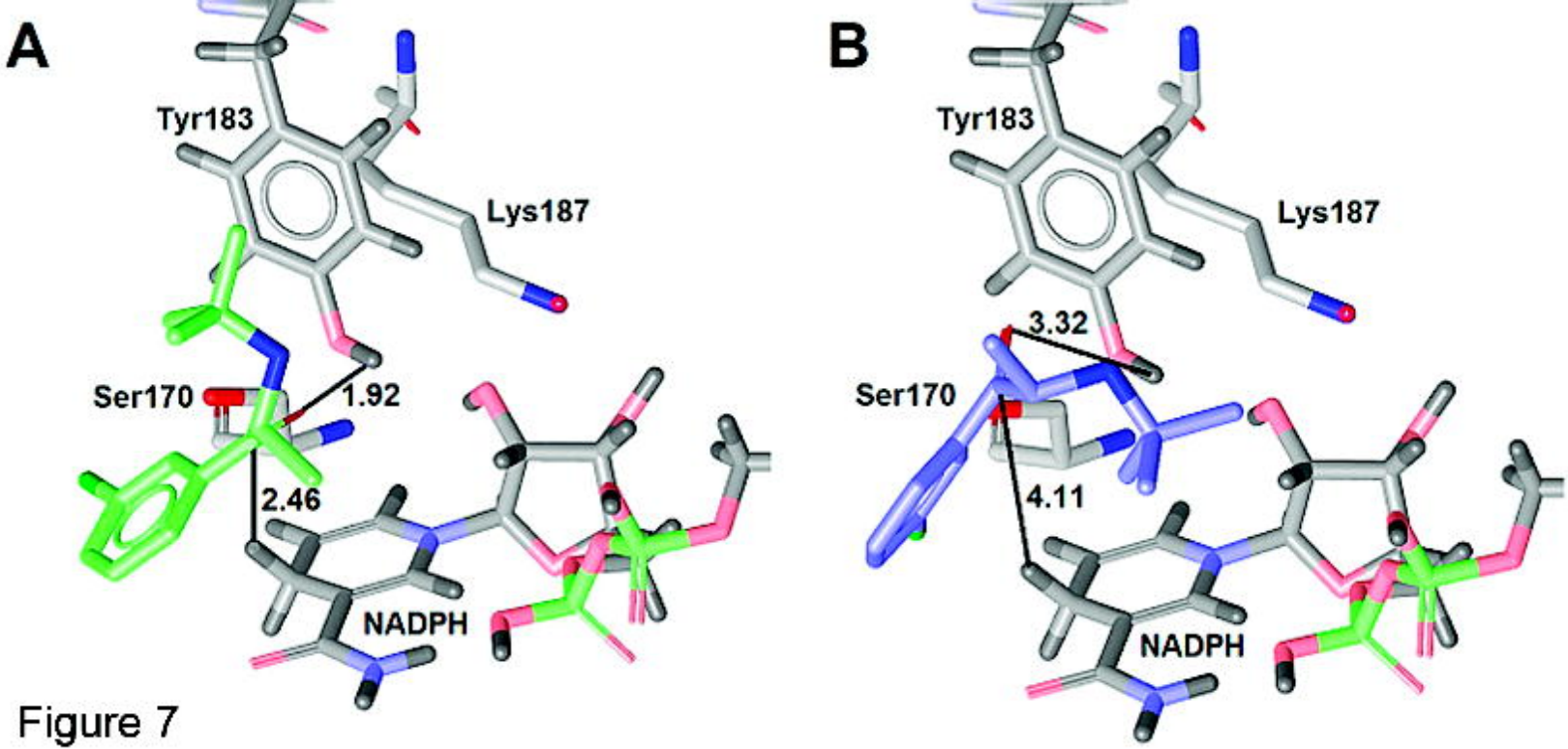
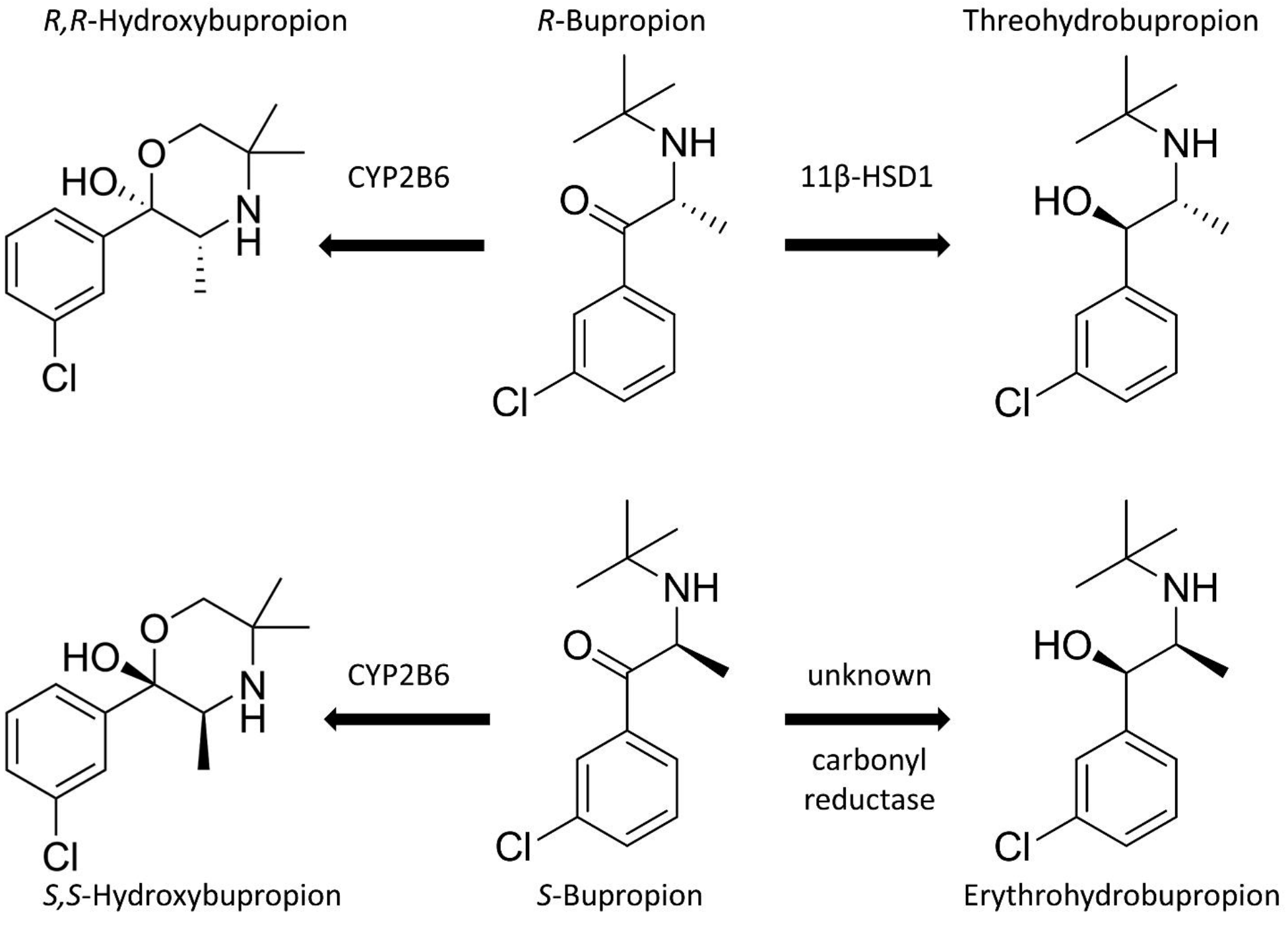

A
Tyr183
Lys187
Ser170
1.92
2.46
NADPH
B
Tyr183
Lys187
3.32
Ser170
4.11
NADPH

Figure 7

Figure 8


R,R-Hydroxybupropion
R-Bupropion
Threohydrobupropion
CYP2B6
11β-HSD1
HO
O
N
H
NH
Cl
S,S-Hydroxybupropion
S-Bupropion
Erythrohydrobupropion
CYP2B6
unknown
carbonyl
reductase