Development of a Rat Plasma and Brain Extracellular Fluid Pharmacokinetic Model for Bupropion and Hydroxybupropion Based on Microdialysis Sampling, and Application to Predict Human Brain Concentrations


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ABSTRACT

Administration of bupropion [(±)-2-(tert-buty lamino)-1-(3-chlorophenyl) propan-1-one] and its preformed active metabolite, hydroxybupropion [(±)-1-(3-chlorophenyl)-2-[(1-hydroxy-2-methyl-2-propanyl)amino]-1-propanone], to rats with measurement of unbound concentrations by quantitative microdialysis sampling of plasma and brain extracellular fluid was used to develop a compartmental pharmacokinetic model to describe the blood–brain barrier transport of both substances. The population model revealed rapid equilibration of both entities across the blood–brain barrier, with resultant steady-state brain extracellular fluid/plasma unbound concentration ratio estimates of 1.9 and 1.7 for bupropion and hydroxybupropion, respectively, which is thus indicative of a net uptake asymmetry. An overshoot of the brain extracellular fluid/plasma unbound concentration ratio at early time points was observed with bupropion; this was modeled as a time-dependent uptake clearance of the drug across the blood–brain barrier. Translation of the model was used to predict bupropion and hydroxybupropion exposure in human brain extracellular fluid after twice-daily administration of 150 mg bupropion. Predicted concentrations indicate that preferential inhibition of the dopamine and norepinephrine transporters by the metabolite, with little to no contribution by bupropion, would be expected at this therapeutic dose. Therefore, these results extend nuclear imaging studies on dopamine transporter occupancy and suggest that inhibition of both transporters contributes significantly to bupropion’s therapeutic efficacy.

Introduction

Bupropion [(±)-2-(tert-buty lamino)-1-(3-chlorophenyl) propan-1-one] was originally introduced as an effective medication for the treatment of depression (Wellbutrin). Among antidepressants, its mechanism is considered atypical in that it has no effects on the serotonergic system; rather, its efficacy in depression has been attributed to its ability to increase dopaminergic and noradrenergic tone, presumably via blockade of the respective synaptic reuptake transporters: the dopamine reuptake transporter (DAT) and the norepinephrine reuptake transporter (NET) (Fava et al., 2005). After its approval for depression, bupropion was also marketed as a smoking cessation aid (Zyban). In addition to its effects on the DAT and NET, it has been suggested that bupropion’s efficacy in nicotine dependence may be associated with alteration of central nicotinic acetylcholine receptor function (Slemmer et al., 2000; Damaj et al., 2004). Single and repeat administration of therapeutic doses of bupropion in humans indicate that bupropion represents a minor fraction of the total bupropion-related plasma exposure, with one of its metabolites, hydroxybupropion [(±)-1-(3-chlorophenyl)-2-[(1-hydroxy-2-methyl-2-propanyl)amino]-1-propanone], representing the predominant circulating entity (Ascher et al., 1995; Jefferson et al., 2005). The relative systemic exposure of this metabolite to bupropion in repeated dosing ranges from 5- to 15-fold (Johnston et al., 2001; Learned-Coughlin et al., 2003; Benowitz et al., 2013). Given this significant difference, it has been suggested that hydroxybupropion, which has similar inhibitory activity at the DAT, but is a more potent NET inhibitor than bupropion, contributes significantly to bupropion’s efficacy (Ascher et al., 1995; Bondarev et al., 2003; Damaj et al., 2004; Lukas et al., 2010). Moreover, the higher exposure to hydroxybupropion may also be contributing to the side effects and adverse events associated with bupropion therapy, particularly seizures observed at higher doses (Wooltorton, 2002).

Use of animal models, particularly mice and rats, to understand the complex pharmacology of bupropion is hampered by the very different disposition of bupropion in animals versus humans. In these rodent species, hydroxybupropion contributes approximately 10% to the total systemic and whole brain exposure observed (Suckow et al., 1986), reflecting a lower systemic exposure relative to humans. Using microdialysis techniques, the brain barrier transport of both bupropion and hydroxybupropion was assessed in the rat model using both mouse and rat species (Dunker and Stratford, 2005).

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which is in complete reversal to the already-noted relative exposures observed in humans. Thus, superimposed upon remaining uncertainty regarding bupropion pharmacodynamics, either directly via inhibition of transporter-mediated reuptake of dopamine and norepinephrine or by indirect effects on these two neurotransmitter systems (Cooper et al., 1994; Dong and Blier, 2001), or via effects on nicotinic acetylcholine receptors (Slemmer et al., 2000; Damaj et al., 2004), the different metabolic disposition in rodents complicates the ability to extrapolate to humans. Although application of brain imaging modalities, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), has received considerable attention (Meyer et al., 2002; Learned-Coughlin et al., 2003; Årgyelán et al., 2005), these approaches cannot distinguish the contributions of bupropion and hydroxybupropion to measured receptor occupancy.

Previous studies have shown that a translational pharmacokinetics (PK)/pharmacodynamics (PD) approach can be of great value to increase understanding of the pharmacology of central nervous system drugs, particularly those with multiple targets and/or with active metabolites. This approach describes plasma-to-brain transfer [blood–brain barrier (BBB) transport], using compartmental or physiologically based pharmacokinetic modeling in animals, with subsequent coupling of measured human systemic PK with the human brain disposition derived from weight-based allometric scaling of the corresponding animal parameters (de Lange, 2013). The method has been used to predict atomoxetine and duloxetine exposure in human brain extracellular fluid (ECF) (Kielbas and Stratford, 2012), risperidone and its metabolite (9-OH-resperidone) receptor occupancy (Kozielska et al., 2012), olanzapine receptor occupancy (Johnson et al., 2011), and receptor occupancy of clozapine and its active metabolite (N-desmethyclozapine) at multiple receptors, based on brain ECF measures using microdialysis (Li et al., 2014). In these examples, predicted exposure and/or occupancy in the human brain after subtherapeutic or therapeutic doses was accordingly congruent with in vitro receptor potency (Kielbas and Stratford, 2012) or with receptor occupancy measured by PET imaging (Johnson et al., 2011; Kozielska et al., 2012; Li et al., 2014). Thus, the objective of our study was to apply this translational approach to predict bupropion and hydroxybupropion exposure in human brain ECF, the pharmacologically relevant biophase for the purported parenchymal membrane targets. To this end, single doses of bupropion and its preformed metabolite were administered to two groups of rats, with subsequent application of population compartmental modeling of measured unbound concentrations from quantitative microdialysis sampling of both plasma and brain ECF. Structural parameters describing brain ECF disposition were then scaled to predict the time course of both entities in human brain ECF.

Materials and Methods

Drugs and Chemicals. Bupropion (racemic) and (2S,3S)-hydroxybupropion hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO) and were used as received. Formulations for administration were prepared on the day of an experiment. Chemicals used in the preparation of microdialysis perfusion buffer and solvents used for high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) analysis were of reagent grade.

Animal Preparation. Adult male Sprague-Dawley rats (280–350 g; Harlan, Horst, The Netherlands) were used for the experiments. The experiments were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were in accordance with Dutch law, and were approved by the Animal Care and Use Committee of the University of Groningen (Groningen, The Netherlands). After arrival, animals were housed in groups of five in polypropylene cages (40 × 50 × 20 cm) with a wire mesh top in a temperature-controlled (22 ± 2°C) and humidity-controlled (55% ± 15%) environment on a 12-hour light cycle (07.00–19.00). After surgery, animals were housed individually (cages 30 × 30 × 30 cm). Standard diet (Diets, RMH-B 2181; ABDiets, Woerden, The Netherlands) and domestic-quality mains water were available ad libitum.

Surgery for implantation of the microdialysis guide cannula was conducted under isoflurane anesthesia (2% with 400 ml/min N2O and 400 ml/min O2), using bupivacaine/epinephrine for local analgesia and finadyne for perioperative/postoperative analgesia. One guide cannula was inserted into the medidial prefrontal cortex to achieve the following probe tip coordinates: anteroposterior, +3.4 mm from bregma; lateral, −0.8 mm from midline; and ventral, −5.0 mm from dura. A second 4.2 cm indwelling cannula was inserted into the jugular vein. Guide cannulae were exteriorized through an incision at the top of the head. Animals were allowed at least 2 days to recover from surgery. MetaQuant Ultra-Slow Flow microdialysis probes (generated cellulose membrane, 4-mm open membrane surface; BrainLink, Groningen, The Netherlands) were inserted 1 day before the experiments.

Drug Administration and Sample Collection. On the day of an experiment, probes were connected with flexible PEEK tubing to a CMA 102 microdialysis pump (CMA Microdialysis, Solna, Sweden) and perfused with a filtered Ringer’s buffer containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgCl2 at a flow rate of 0.12 µl/min (CMA 142 pump). The slow flow design of this probe maximizes sample recovery (Cremers et al., 2009), which was 91% for bupropion and 100% for hydroxybupropion, as determined from in vitro recovery experiments. Ultrapurified water was perfused through the dilution inlet of the probe at a flow rate of 0.8 µl/min; thus, the total dialysate flow rate was 0.92 µl/min. After initiating flow, probes were allowed to stabilize for 2 hours prior to administration of a single subcutaneous dose of bupropion (10 mg/kg, n = 7 with partial overlap for each matrix) or hydroxybupropion (2 mg/kg, n = 4), both dissolved in 0.9% NaCl at 2 mg/ml. Dialysates from the brain and jugular vein were collected every 30 minutes starting 1 hour prior to administration and continuing for 360 minutes after drug administration. Dialysate samples were stored at −80°C until time of analysis.

Sample Collection. Concentrations of compounds were measured in dialysate collected from plasma and brain ECF probes using HPLC with MS/MS detection. An aliquot of 12 µl was taken from each MetaQuant dialysate sample (27.6 µl total volume) and mixed with 4 µl internal standard solution (fenfluramine). Of this mixture, 10 µl was injected into the HPLC system by an automated sample injector (SIL-20AD; Shimadzu, Kyoto, Japan). Chromatographic separation of the compounds was performed on a reversed phase column (100 × 3.0 mm, 2.5 µm particle size; Phenomenex, Torrance, CA) held at a temperature of 35°C in a gradient elution run, using eluent B (acetonicitrile) in eluent A (10 mM ammonium formate in ultrapurified water, pH 4) at a flow rate of 0.3 ml/min. The gradient profile was as follows: 0% B from 0 to 4 minutes, 40%–100% B from 4 to 5.5 minutes, remaining at 100% B until 6 minutes, and returning to 0% B from 6 to 6.5 minutes. The mass spectrometry analyses were performed using an API 4000 MS/MS system consisting of an API-4000 MS/MS detector and a Turbo ION Spray interface (both from Applied Biosystems, Foster City, CA). The acquisitions were performed in positive ionization mode, with ionization spray voltage set at 5.5 kV and a probe temperature of 500°C. The instrument was operated in multiple reaction monitoring mode. Multiple reaction monitoring transitions were 239.9 to 183.9 for bupropion and 256.0 to 166.0 for hydroxybupropion. Suitable in-run calibration curves were fitted using weighted (1/x) regression, and the sample concentrations were determined using these calibration curves. Standard concentrations ranged from 0.05 to 50.0 nM. Accuracy was verified by quality-control samples after each sample series. Concentrations were calculated with the Analyst data system (Applied Biosystems).

Pharmacokinetic Analysis. All pharmacokinetic analyses were conducted with Phoenix NLME 1.3 (Pharsight Corporation, Certara, L.P., Princeton, NJ). A population modeling approach was used for both bupropion and hydroxybupropion using first-order conditional estimation. Initial models in plasma were built for each compound based on their corresponding dose group (bupropion and preformed hydroxybupropion). Different model structures were evaluated for each compound (bupropion and hydroxybupropion). Suitable in-run calibration curves were fitted using weighted (1/x) regression, and the sample concentrations were determined using these calibration curves. Standard concentrations ranged from 0.05 to 50.0 nM. Accuracy was verified by quality-control samples after each sample series. Concentrations were calculated with the Analyst data system (Applied Biosystems).
parameters, was used to compare models differing by more than one parameter. After optimization of the plasma model unique to each dose group, brain ECF concentrations were incorporated as a peripheral compartment. Transfer characteristics between the plasma and brain were modeled using either a single intercompartmental clearance (Q) or separate parameters for the uptake and efflux apparent distributional clearances (CL\text{in} and CL\text{out}, respectively). The unbound volume of distribution of bupropion in the brain (V\text{b,HB}) was tested with versus without fixing this parameter to a literature-reported value of 8 ml/g brain (Fridén et al., 2011). For hydroxybupropion, a computational approach was used to estimate its volume of distribution in the brain (V\text{b,HB}) (Speafico and Jacobson, 2013). Based on this approach, a value of 8 ml/g brain was used when this parameter was fixed. In addition to minimization of OFV, the combined plasma-brain ECF model selection also included stability of the plasma model—specific estimates upon incorporation of the brain ECF compartment.

In parallel with development of the plasma-brain models for each compound, a combined plasma model that incorporated hydroxybupropion concentrations observed after both bupropion administration (formed metabolite) and hydroxybupropion administration (preformed metabolite) was developed. Linkage of observed after both bupropion administration (formed metabolite) and hydroxybupropion concentrations from the two multiple dose studies after correcting for plasma protein binding of 77% (Johnston et al., 2002). Both bupropion and hydroxybupropion had different bioavailability, 2) that structures were investigated. These included 1) the possibility that bupropion prior to it reaching the systemic circulation (the so-called "first-pass effect"), 3) a model that included a presystemic component to bupropion metabolism, and 4) a model that incorporated clearance of formed hydroxybupropion prior to it reaching the systemic circulation (the so-called "sequential first-pass elimination of the formed metabolite" model; Pang and Gillette, 1979). Neither different bioavailability nor the various clearance models could describe the observed alteration in

Results

Subcutaneous administration of a 10-mg/kg dose of bupropion resulted in both parent drug and formed metabolite exposure in plasma and brain ECF (Table 1). The hydroxybupropion area under the curve (AUC\text{C0-\infty}) as a percentage of bupropion exposure was 6% ± 0.6% (n = 6) in plasma and 4% ± 0.3% in brain ECF (n = 6). The relative exposure in plasma agrees reasonably well with the 13% reported after a 40-mg/kg intraperitoneal dose to rats (Suckow et al., 1986). In addition, a recent microdialysis study (Yenicielli et al., 2011) measured comparable AUCs for bupropion (within 2-fold) and hydroxybupropion (within 3-fold) in brain ECF after a 10-mg/kg intraperitoneal dose to rats. In our study, for both plasma and brain matrices, a decline of the metabolite proceeded in parallel with that of bupropion; in addition, the terminal half-life in brain ECF was similar to the plasma for each compound (Table 1). With respect to preformed hydroxybupropion administration (2 mg/kg), its terminal half-life in both plasma and brain ECF was approximately 50% of its half-life observed after bupropion administration.

The ratio of brain ECF concentration to unbound plasma concentration (K\text{p,uu}) was on average above 1 at all times for bupropion and preformed hydroxybupropion (Fig. 1). AUC ratios, shown in Table 1, indicate a 2.1-fold asymmetry for bupropion and slightly lower values for both preformed and formed hydroxybupropion.

Compartmental model structure definition for bupropion in plasma and brain ECF indicated that a one-compartment model provided the best description of concentration time course in both matrices, in agreement with visual inspection of monoexponential decline in individual animals (Fig. 2). The same was true for hydroxybupropion in both matrices, regardless of its disposition as formed or preformed metabolite. Estimation of bupropion and of preformed metabolite bioavailability resulted in population estimates > 95%. However, since precision of these estimates, as well as of the corresponding clearance and volume of distribution (V\text{D}) estimates was poor, bioavailability was fixed to 0.95 for bupropion to account for a small first-pass effect and to 1 for preformed hydroxybupropion. With respect to the description of plasma-to-brain ECF transfer, for bupropion and for its metabolite, estimation of CL\text{in} and CL\text{out} provided clearly superior results (minimization of OFV) relative to modeling this transfer as a single intercompartmental clearance. Importantly, an attempt was made to estimate the unbound brain volume of distribution for both bupropion (V\text{b,HB}) and hydroxybupropion (V\text{b,HB}) however, it was necessary to fix this parameter for both compounds to get reliable estimates of the distributional clearances.

The initial approach to combine the plasma disposition of bupropion and hydroxybupropion after their subcutaneous administration was to fix the parameter estimates obtained from modeling the two administration groups separately to define the formation clearance for the formed hydroxybupropion. This approach led to a formation clearance of approximately 3.5 ml/min. Subsequent population modeling of plasma data simultaneously from both groups of animals, using this estimate and without fixing the parameters for the two compounds, resulted in model nonconvergence. Therefore, various plasma model structures were investigated. These included 1) the possibility that bupropion and hydroxybupropion had different bioavailability, 2) that bupropion was altering the clearance or V\text{D} of the formed metabolite, 3) a model that included a presystemic component to bupropion metabolism, and 4) a model that incorporated clearance of formed hydroxybupropion prior to it reaching the systemic circulation (the so-called "sequential first-pass elimination of the formed metabolite" model; Pang and Gillette, 1979). Neither different bioavailability nor the various clearance models could describe the observed alteration in

Human Exposure Simulations. The final plasma-brain ECF model was scaled to predict steady-state human brain ECF exposure to bupropion and hydroxybupropion. A 150-mg dose of the extended-release (SR) bupropion product administered twice daily was selected based on studies of bupropion PK using this formulation and dose frequency (Johnston et al., 2001; Learned-Coughlin et al., 2003; Benowitz et al., 2013). Bupropion pharmacokinetic parameters used for the simulations were based on these studies and corrected for plasma protein binding of 85% (Jefferson et al., 2005). These were 17.5 l/min for CL/F and 12,400 liters for V/F. An absorption rate constant (k\text{a}) of 0.01/min, which yielded a T\text{max} of 3 hours, the reported value for the SR formulation (Jefferson et al., 2005), was used. Pharmacokinetic parameters for formed hydroxybupropion were adjusted to provide an average unbound steady-state concentration of 500 nM. This concentration was between the reported average concentrations from the two multiple dose studies after correcting for hydroxybupropion plasma protein binding of 77% (Johnston et al., 2002). Both bupropion and hydroxybupropion pharmacokinetics are linear after long-term bupropion administration of 300–400 mg/d (Jefferson et al., 2005). Weight-based allometric scaling of the plasma-brain ECF distributional clearance (CL) and brain volumes was used to derive the corresponding human brain parameters, using a rat brain weight of 2.45 g (1.8 g/250 g body weight; Davies and Morris, 1993) and a human brain weight of 1.35 kg. The exponential factors were 0.75 for CL and 1.0 for brain distribution volume (V\text{b,HB}) (Sharma and McNiel, 2009). A total of 1000 simulations were conducted.

Data Presentation. Rat plasma and brain ECF matrix concentration data are presented as unbound concentrations, and the corresponding time points are midpoints of a given 30-minute collection interval. All pharmacokinetic parameters are presented as the unbound estimates.
formed metabolite disposition relative to the preformed metabolite. A model that invoked altered hydroxybupropion VD in the presence of bupropion was also unable to describe formed metabolite disposition. Ultimately, structure 3 above, a model that incorporated a presystemic (first-pass) component of bupropion conversion to hydroxybupropion, was selected as the final plasma model. Table 2 provides a summary of the final population model parameter estimates, as well as estimates of BAV for several of the parameters, and residual error. Also shown are the results of a nonparametric bootstrap to evaluate parameter stability in the plasma model. Figure 3 summarizes individual and population predicted concentrations versus the observed concentrations for both bupropion and hydroxybupropion in plasma, as well as the conditional weighted residuals distributions relative to both time and the population predicted concentrations.

The final plasma-brain ECF model, which was based on fixed plasma parameter estimates with simultaneous analysis of brain ECF concentrations from both administration arms, is shown in Fig. 2. The assumption made in fixing the plasma estimates to derive the brain ECF estimates is that brain kinetics do not influence plasma kinetics. This assumption is supported by the much larger systemic volumes of distribution relative to those in the brain, as well as the two administration arm plasma-brain ECF–specific models that showed stability of the plasma estimates upon addition of the brain ECF component. The final model also incorporated a time-dependent component to CLinb, expressed as CLinb = CLinb0 – slp × time, where CLinb decreases with time (slope) up to 195 minutes and is constant thereafter, and CLinb = CLinb0 when time = 0. A similar approach was recently used to describe time dependence in citalopram absorption in rats (Velez de Mendizabal et al., 2015) and was conceived in our case based on the observation in four of five rats of an overshoot in Kp,uu in the first half of the time course relative to the eventual Kp,uu,ss (Fig. 1).

Table 3 provides a summary of population parameter estimates as well as estimates of BAV for the various plasma-to-brain ECF distributional clearances and proportional residual error.

Figure 4 summarizes individual and population predicted concentrations versus the observed concentrations for both bupropion and hydroxybupropion in brain ECF, as well as the conditional weighted residual distributions relative to both time and the brain ECF population predicted concentrations. Figure 5 summarizes the results of the visual predictive check for both compounds in plasma and brain ECF. Results from an evaluation of parameter precision in the final combined plasma and brain ECF model based on nonparametric bootstrap analysis are summarized in Table 3.

Figure 6 presents the predicted steady-state exposures in human plasma and brain ECF from 0 to 12 hours for bupropion and hydroxybupropion after twice-daily administration of the 150-mg extended-release bupropion SR tablet. The median maximum bupropion plasma concentration was 59 nM, occurring at 3 hours, which is the commonly observed peak time for the bupropion SR product (Jefferson et al., 2005), and the minimum concentration was 25 nM. These concentrations agree well with the reported maximum and minimum concentrations of 59 nM and 16 nM, respectively (Johnston et al., 2001), when corrected for bupropion’s plasma protein binding of 85% (Jefferson et al., 2005). Median predicted hydroxybupropion plasma concentrations varied little over the 12-hour time course (from 729 nM at the end of the dose interval to a peak of 798 nM 6 hours after administration) and agree well with reported steady-state hydroxybupropion plasma concentrations (Johnston et al., 2001; Learned-Coughlin et al., 2003) when corrected for hydroxybupropion’s plasma protein binding of 77% (Johnston et al., 2002). A comparison with reported IC50 values for DAT and NET (Damaj et al., 2004; Lukas et al., 2010) shows that unbound brain concentrations of hydroxybupropion after twice-daily administration of 150 mg bupropion SR exceed its IC50 values for DAT (630 nM) and NET (241 nM) over the entire time course, whereas bupropion concentrations were substantially lower than reported IC50s for the two transporters (660 nM and 1850 nM, respectively) at all times.

**Discussion**

Incorporation of a preformed hydroxybupropion administration group into the study design revealed that disposition of the formed metabolite was rate-limited by bupropion kinetics in both plasma and brain ECF. Coupled with the observation that preformed metabolite half-life in brain ECF was similar to that observed in plasma, these findings indicate that metabolite uptake and egress across the BBB and its intrabrain distribution were faster than metabolite systemic disposition, both with respect to its formation and subsequent elimination. Parenthetically, in humans, given that hydroxybupropion and bupropion have a similar half-life after bupropion administration (Sweet et al., 1995; Hsu et al., 1997), it is possible that hydroxybupropion disposition, at least in plasma, is also rate-limited by bupropion. In our rat study, comparing systemic AUCs of formed metabolite to bupropion and correcting for differences in clearance between bupropion and that of the preformed metabolite (Rowland and Tozer, 2011), the fraction of bupropion metabolized to hydroxybupropion was 1.1%. In the compartmental model, the ratio of formation clearance to total clearance of bupropion was 0.4%. The difference is attributed to a small pre systemic clearance component prior to bupropion systemic absorption. Albeit small, this first-pass effect is surprising given that bupropion was administered subcutaneously. In response to nonconvergence of the combined formed and preformed metabolite plasma model that did not include this first-pass effect, alternative explanations were investigated: 1) bupropion-mediated alteration of formed metabolite disposition (altered clearance or Vp) or 2) different bioavailability for the two dose groups. These alternative structures were found unable to generate a model that included the two dose groups. In addition, the possibilities that hydroxybupropion probe recovery was enhanced in the presence of
Fig. 1. Bupropion and hydroxybupropion unbound concentration ratios of brain ECF to plasma ($K_{p,uu}$) in individual rats. The top panel presents bupropion ratios after a 10-mg/kg subcutaneous dose of bupropion, whereas the middle panel presents hydroxybupropion ratios observed in the same group of rats (formed metabolite case). The bottom panel presents hydroxybupropion ratios after a 2-mg/kg dose of the metabolite in a different group of rats by the same administration route (preformed metabolite case). The x-axis represents a given animal, with each point representing a sample time arranged in ascending order from left to right starting at 15 minutes and ending at 345 minutes in 30-minute increments. The solid horizontal line in each panel represents the plasma-to-brain equilibration model–predicted equilibrium $K_{p,uu}$ value reported in Table 2.
bupropion or of bias in the analysis of the metabolite in the presence of bupropion were considered and discounted. At this point, the cause of the presumed presystemic component is not understood.

The observation that bupropion brain kinetics paralleled those in plasma indicates that, like hydroxybupropion, bupropion disposition in brain was faster than its overall (systemic) disposition in the rat. In fact, brain equilibration half-life for each compound (based on the equation \( t_{1/2,\text{equil}} = \frac{V_b}{\text{CL}_{\text{out}} \cdot \ln 2} \); Liu et al., 2005) was only 1.4 minutes and 4.6 minutes for bupropion and hydroxybupropion, respectively. As shown in Fig. 1, in four of the five animals receiving bupropion, there was a distinct peak in \( K_{pu} \) for bupropion occurring by the second collection interval. A significant improvement in model performance (AIC and

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BAV (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bupropion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{CL}_{B/F} ) (ml/min)</td>
<td>316 (10.6)</td>
<td>313 (270–366)</td>
</tr>
<tr>
<td>( \text{V}_{pu,B/F} ) (ml)</td>
<td>56244 (19.8)</td>
<td>55,836 (43,053–79,639)</td>
</tr>
<tr>
<td>( k_{ah} ) (1/min)</td>
<td>0.0355 (14.8)</td>
<td>0.0357 (0.0285–0.0485)</td>
</tr>
<tr>
<td>( \text{T}_{lag} ) (min)</td>
<td>11.8 (2.3)</td>
<td>11.8 (11.4–12.4)</td>
</tr>
<tr>
<td>( \text{CL}_{F} ) (ml/min)</td>
<td>1.4 (29.9)</td>
<td>1.4 (0.7–2.5)</td>
</tr>
<tr>
<td>( k_{fp} ) (1/min)</td>
<td>0.00027 (26.6)</td>
<td>0.00027 (0.00017–0.00045)</td>
</tr>
<tr>
<td>( F_B )</td>
<td>0.95 (fixed)</td>
<td>11.8 (12.6)</td>
</tr>
<tr>
<td><strong>Hydroxybupropion</strong></td>
<td></td>
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</tr>
<tr>
<td>( \text{CL}_{HB/F} ) (ml/min)</td>
<td>67.3 (17.2)</td>
<td>67.4 (46.7–93.1)</td>
</tr>
<tr>
<td>( \text{V}_{pu,HB/F} ) (ml)</td>
<td>5329 (16.9)</td>
<td>5288 (3867–6989)</td>
</tr>
<tr>
<td>( k_{ah} ) (1/min)</td>
<td>0.1 (fixed)</td>
<td>0.047 (12.9)</td>
</tr>
<tr>
<td>( F_B )</td>
<td>1 (fixed)</td>
<td>11.8 (3.4)</td>
</tr>
<tr>
<td><strong>Residual error (proportional)</strong></td>
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<tr>
<td>Bupropion-plasma</td>
<td>0.107 (13.7)</td>
<td>0.107 (0.083–0.132)</td>
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<tr>
<td>Hydroxybupropion-plasma</td>
<td>0.139 (10.9)</td>
<td>0.160 (0.132–0.185)</td>
</tr>
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</table>

B, bupropion; \( \text{CL}_{F} \), apparent elimination clearance for bupropion or hydroxybupropion; \( \text{CL}_{F} \), hydroxybupropion formation clearance after bupropion administration; \( F_B \), bioavailability fraction; HB, hydroxybupropion; \( k_{ah} \), first-order rate constant for absorption; \( k_{fp} \), first order rate constant for presystemic metabolism of bupropion to hydroxybupropion; RSE, relative standard error of the estimate; \( \text{T}_{lag} \), lag time for bupropion absorption; \( \text{V}_{pu} \), apparent brain volume of distribution.

Bupropion and Hydroxybupropion Brain Pharmacokinetics

**Fig. 2.** Compartmental pharmacokinetic model used to describe bupropion, and its formed and preformed metabolite absorption and disposition in rats. Terms with a subscript containing B represent bupropion, whereas those with HB, hydroxybupropion. \( C_{\text{ECF}} \), unbound brain ECF concentration; \( C_{\text{u,p}} \), unbound plasma concentration; \( \text{CL}_{F} \), apparent elimination clearance; \( \text{CL}_{\text{out}} \), apparent distributional clearance; \( k_a \), first-order rate constant for presystemic conversion of bupropion to hydroxybupropion; \( \text{T}_{lag} \), lag time for bupropion absorption; \( \text{V}_{pu} \), apparent brain volume of distribution; \( \text{V}_{pu} \), apparent systemic volume of distribution.
goodness-of-fit plots) was achieved by incorporating a time dependency in $CL_{in,B}$, with this decreasing over time. In the final population model, the ratio of $CL_{in}/CL_{out}$ was 1.9 and 1.7 (relative standard error of the estimate = 5.6% and 6.1%) for bupropion and hydroxybupropion, respectively, both in good agreement with the AUC ratio estimates in Table 1. The model did not include brain ECF bulk flow, since the estimated value of $0.18$ to $0.29 \text{ ml/min} \times \text{g brain}$ (Szentistványi et al., 1984) is well below 1% of the $CL_{out}$ estimates and thus is a negligible contributing factor to brain clearance. The utility of modeling plasma and brain distribution across the BBB is that this approach converts an observation ($K_{puu}$) into parameters ($CL_{in}$ and $CL_{out}$) that correlate with physiologic carrier-mediated processes that are potentially scalable between species (Qiu et al., 2014), and whose function could be sensitive to intersubject variability caused by disease or genetic variability as well as drug–drug and drug–metabolite interactions.

The experimentally obtained $pK_a$ of bupropion has been estimated at 7.9 (Gondaliya and Pundarikakshudu, 2003) and 8.6 (Fridén et al., 2011). Taking the average of these two values, and assuming that brain ECF is 0.1 log unit more acidic than plasma (pH 7.3 versus pH 7.4; Fridén et al., 2011), results in a predicted diffusional equilibrium preference on the brain side of 1.01, which supports that the $CL_{in}/CL_{out}$ ratios of approximately 2 are due to an uptake transporter for bupropion and hydroxybupropion at the BBB. The net uptake asymmetry observed is similar to oxycodone BBB transport in rats, (Boström et al., 2006), which showed a net 3-fold asymmetry favoring rat brain ECF relative to unbound plasma concentrations. Subsequent in vitro

**Fig. 3.** Model diagnostic plots for plasma. (A and B) Individual predicted concentrations versus observed concentrations for bupropion and hydroxybupropion, respectively. The solid line is the line of unity. (C and D) Population predicted concentrations versus observed concentrations for bupropion and hydroxybupropion, respectively. (E and F) Conditional weighted residuals versus time for bupropion and hydroxybupropion, respectively. (G and H) Conditional weighted residuals versus population predicted concentrations for bupropion and hydroxybupropion, respectively. CWRES, conditional weighted residual; DV, concentration; IPRED, individual predicted; PRED, population predicted.

### Table 3

Brain ECF bupropion and hydroxybupropion population pharmacokinetic parameter estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BAV (RSE%)</th>
<th>Bootstrap Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>Bupropion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CL_{in,B0}/F$ (ml/min)</td>
<td>18.3 (11.2)</td>
<td>3.4 (1.9)</td>
<td>18.6 (16.9–43.8)</td>
</tr>
<tr>
<td>$CL_{out,B}/F$ (ml/min)</td>
<td>5.4 (16.5)</td>
<td>5.6 (0.3)</td>
<td>5.5 (4.4–16.2)</td>
</tr>
<tr>
<td>$V_{b,B}/F$ (ml)</td>
<td>10.67 (fixed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Slp$</td>
<td>0.04 (fixed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxybupropion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CL_{in,HB}/F$ (ml/min)</td>
<td>1.3 (30.1)</td>
<td>132.9 (24.5)</td>
<td>1.4 (0.9–1.9)</td>
</tr>
<tr>
<td>$CL_{out,HB}/F$ (ml/min)</td>
<td>0.8 (26.9)</td>
<td>99.1 (15.9)</td>
<td>0.8 (0.5–1.2)</td>
</tr>
<tr>
<td>$V_{b,HB}/F$ (ml)</td>
<td>5.33 (fixed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error (proportional)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bupropion-brain</td>
<td>0.165 (8.8)</td>
<td>0.166 (0.143–0.188)</td>
<td></td>
</tr>
<tr>
<td>Hydroxybupropion-brain</td>
<td>0.163 (6.3)</td>
<td>0.163 (0.146–0.178)</td>
<td></td>
</tr>
</tbody>
</table>

B, bupropion; $CL_{in,B0}/F$, apparent influx clearance for bupropion at initial time; $CL_{in,HB}$, apparent influx clearance for hydroxybupropion; $CL_{out}$, apparent efflux clearance; HB, hydroxybupropion; RSE, relative standard error of the estimate; $slp$, slope relating time-dependent change in $CL_{in,B}$; $V_{b}$, apparent brain volume of distribution.
studies with oxycodone (Okura et al., 2008)—as well as more recent in vitro transport or in situ brain perfusion studies with several other low molecular weight weakly basic drugs, such as apomorphine (Okura et al., 2014), clonidine (André et al., 2009), codeine (Fischer et al., 2010), diphenhydramine (Shimomura et al., 2013), and nicotine (Cisternino et al., 2013)—implicate a pH-dependent proton-coupled antiporter at the BBB (Gharavi et al., 2015). It is possible that this transporter may also apply to bupropion and hydroxybupropion. Moreover, the proton-coupled antiporter has been suggested to be capable of bidirectional transport (Cisternino et al., 2013). It is possible that the overshoot in bupropion $K_{p,uu}$, which was modeled as a time-dependent distributional clearance, may have been due to eventual replacement of protons by bupropion and/or hydroxybupropion on the abluminal side. This may have resulted in a decrease in CL$_{uu}$ over time due to reduced function, or just to loss of the proton gradient over time, much like occurs with proton-coupled dipeptide transport in the small intestine (Ganapathy and Leibach, 1983). Interestingly, a similar time-dependent $K_{p,uu}$ was observed with oxycodone (Boström et al., 2006). It is possible that the overshoot observed with bupropion is due to an experimental artifact (namely, a decline in probe recovery of bupropion after administration and lasting for 2 to 3 hours, at which point it reached a constant value). As is standard practice in microdialysis research, probes in this study were inserted 24 hours before an experiment and perfused for 2 hours prior to drug administration to

![Fig. 4. Model diagnostic plots for brain ECF.](image)

(A and B) Individual predicted concentrations versus observed concentrations for bupropion and hydroxybupropion, respectively. The solid line is the line of unity. (C and D) Population predicted concentrations versus observed concentrations for bupropion and hydroxybupropion, respectively. (E and F) Conditional weighted residuals versus time for bupropion and hydroxybupropion, respectively. (G and H) Conditional weighted residuals versus population predicted concentrations for bupropion and hydroxybupropion, respectively. DV, concentration; IPRED, individual predicted; PRED, population predicted; WRES, weighted residual.

![Fig. 5. Visual predictive checks for plasma (top) and brain ECF (bottom).](image)

The left plot in each panel represents bupropion after a 10-mg/kg dose bupropion, the middle plot represents formed hydroxybupropion after the 10-mg/kg bupropion dose, and the right plot in each panel represents preformed hydroxybupropion after a 2-mg/kg dose of the metabolite. The solid line in each plot represents the median of the observed concentrations, the dashed line represents the median predicted concentrations, and the two dotted lines represent the 5% and 95% limits of the predicted 90% confidence intervals of the median. Individual observed concentrations are shown as the open circles.
reduce the possibility for such an artifact. At a minimum, our observations point to additional studies to develop a better mechanistic understanding of bupropion and hydroxybupropion transport across the BBB.

Bupropion’s effects in rodent models of depression have been linked to its ability to increase dopamine and norepinephrine concentrations in brain ECF, as determined using microdialysis sampling (Nomikos et al., 1989, 1992). More recently, Li et al. (2002) evaluated the effect of a 10-mg/kg bupropion subcutaneous dose on dopamine and norepinephrine levels in the ECF of medial prefrontal cortex of rats, thus employing the same dose, route, and microdialysis probe placement as used in our study. In the study by Li et al. (2002), the increase in dopamine peaked at 262% over baseline 90 minutes after administration. A dopamine increase at that time is in accord with the median peak bupropion concentration of 504 nM observed during the 60- to 90-minute collection interval (Fig. 5), and its proximity to its reported IC₅₀ at DAT of 550–660 nM (Damaj et al., 2004; Lukas et al., 2010).

Although hydroxybupropion also possesses inhibitory activity at DAT in the same range (790 nM) in rats, its contribution to increasing extracellular dopamine would be expected to be minimal, given the median concentration of 22 nM observed. By contrast, hydroxybupropion exposure in plasma in humans is approximately an order of magnitude greater than bupropion (Johnston et al., 2001; Learned-Coughlin et al., 2003; Benowitz et al., 2013). Translation of the rat bupropion and hydroxybupropion plasma-brain distributional clearances to humans suggests that monoamine contributions to bupropion’s antidepressant effects via monoamine reuptake inhibition are due to hydroxybupropion. Median hydroxybupropion brain ECF concentrations are similar to its DAT IC₅₀ and are approximately 3-fold above its NET IC₅₀ (Fig. 6), whereas peak bupropion brain ECF concentrations are approximately 7-fold below the DAT IC₅₀ and are even more so relative to that for NET. Importantly, our predictions of the much larger exposure to the metabolite in brain ECF agree with its reported 7-fold higher concentrations than bupropion in human CSF (Cooper et al., 1994). Receptor occupancy studies using PET (Meyer et al., 2002; Learned-Coughlin et al., 2003) or SPECT (Argyelän et al., 2005) tracers specific for DAT, and after attainment of steady-state kinetics based on several days of dosing 150 mg of the SR formulation twice daily, indicate modest occupancy of around 20% in both patients with and without depression. In one of these studies (Learned-Coughlin et al., 2003), DAT occupancy was sustained for at least 24 hours. Our predicted sustained exposure to hydroxybupropion (Fig. 6) agrees with the reported sustained occupancy at this transporter and suggests that it was due to the metabolite. However, the low occupancy reported in these studies is below that which our predictions would suggest (approximately 50%) based on the simulated concentrations being similar to the reported IC₅₀ for hydroxybupropion at this transporter (630 nM; Lukas et al., 2010). This difference identifies the need for studies to investigate the mechanism of bupropion and hydroxybupropion transport across blood–brain surrogate models and, if a transporter is involved, to apply mass spectrometry–based proteomics to support interspecies scaling (Qiu et al., 2014). The predicted exposures for bupropion and hydroxybupropion, together with their IC₅₀ values for NET, indicate that, if norepinephrine levels are increased in humans via NET inhibition, this would largely be attributed to hydroxybupropion, and not bupropion.

In conclusion, a pharmacokinetic model that predicts bupropion and hydroxybupropion concentrations in brain ECF of rats has been developed. The model asserts a carrier-mediated mechanism contributes to the plasma to brain transfer of both compounds. Similar to what has been done with atomoxetine and duloxetine (Kiellbas and Stratford, 2012) and clozapine and its active metabolite, N-desmethylclozapine...
(Li et al., 2014), to translate rat BBB kinetics to humans to predict human brain exposure, our approach predicts that the dopamine transporter occupancy observed in humans using PET and SPECT is due to hydroxybupropion and that the metabolite is responsible for a direct effect on increasing synaptic dopamine and norepinephrine via DAT and NET inhibition, respectively.

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Conducted experiments: Flik, Folgering.
Performed data analysis: Stratford.
Wrote or contributed to the writing of the manuscript: Cremers, Flik, Folgering, Rollema, Stratford.

References
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