Published in final edited form as:

AAPS J. ; 20(1): 21. doi:10.1208/s12248-017-0180-7.

The Drug of Abuse Gamma-Hydroxybutyric Acid Exhibits Tissue-Specific Nonlinear Distribution

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Abstract

The drug of abuse γ -hydroxybutyric acid (GHB) demonstrates complex toxicokinetics with dose-dependent metabolic and renal clearance. GHB is a substrate of monocarboxylate transporters (MCTs) which are responsible for the saturable renal reabsorption of GHB. MCT expression is observed in many tissues and therefore may impact the tissue distribution of GHB. The objective of the present study was to evaluate the tissue distribution kinetics of GHB at supratherapeutic doses. GHB (400, 600, and 800 mg/kg iv) or GHB 600 mg/kg plus L-lactate (330 mg/kg iv bolus followed by 121 mg/kg/h infusion) was administered to rats and blood and tissues were collected for up to 330 min post-dose. K_p values for GHB varied in both a tissue- and dose-dependent manner and were less than 0.5 (except in the kidney). Nonlinear partitioning was observed in the liver (0.06 at 400 mg/kg to 0.30 at 800 mg/kg), kidney (0.62 at 400 mg/kg to 0.98 at 800 mg/kg), and heart (0.15 at 400 mg/kg to 0.29 at 800 mg/kg), with K_p values increasing with dose consistent with saturation of transporter-mediated efflux. In contrast, lung partitioning decreased in a dose-dependent manner (0.43 at 400 mg/kg to 0.25 at 800 mg/kg) suggesting saturation of active uptake. L-lactate administration decreased K_p values in liver, striatum, and hippocampus and increased K_p values in lung and spleen. GHB demonstrates tissue-specific nonlinear distribution consistent with the involvement of monocarboxylate transporters. These observed complexities are likely due to the involvement of MCT1 and 4 with different affinities and directionality for GHB transport.

Keywords

Gamma-hydroxybutyric acid; Monocarboxylate transporters; Toxicokinetics

INTRODUCTION

Gamma-hydroxybutyric acid (GHB), a monocarboxylic acid, is formed endogenously from γ -aminobutyric acid (GABA) within the brain (1). A putative GHB receptor has been

identified within the brain of rats and humans (2–4) and is postulated to be involved in GHB's endogenous function, GABA_B receptor-mediated pathways are thought to mediate the pharmacological and toxicological effects of GHB (5,6). Clinically, GHB is used in the treatment of narcolepsy with cataplexy in the USA and Europe (marketed in the USA as Xyrem®; Jazz Pharmaceuticals, Palo Alto, CA) and alcohol withdrawal in Europe (7). However, abuse of GHB and its precursors, 1,4-Butanediol and γ -butyrolactone, is widespread due to the sedative/hypnotic, euphoric, and growth hormone releasing effects of GHB (8,9). The toxicological effects of a GHB overdose are characterized by cardiovascular effects and central nervous system depression that can lead to coma and death (10). There are currently no therapeutic interventions clinically available for the treatment of GHB overdose.

GHB demonstrates nonlinear toxicokinetics due to capacity-limited metabolism (11,12), oral absorption (13), and renal reabsorption (14,15). Metabolic clearance is saturated and renal clearance becomes a significant route of elimination at supratherapeutic doses of GHB. Inhibition of active renal reabsorption of GHB further increases its renal clearance (14). Transport of GHB within renal proximal tubules is mediated by the pH- and sodium-dependent families of monocarboxylate transporters (MCTs and SMCTs) (14,16). Physiologically, MCTs 1–4 and SMCTs are involved in the transport of monocarboxylic acids, such as L-lactate, pyruvate, and butyrate which are vital to cellular function. MCT1 (SLC16A1) is ubiquitously expressed throughout the body with MCT 2 (SLC16A7) and MCT4 (SLC16A3) expressed in many tissues with varied expression levels (17). In addition to renal reabsorption, MCT-mediated transport of GHB has been demonstrated in the intestine (13,18), within the brain (19), and at the blood-brain barrier (20,21). SMCT1 (SL5A8) and SMCT2 (SLC5A12) demonstrate a more restricted distribution with expression observed in the kidney, intestine, brain, and thyroid (22).

Previous studies have demonstrated concentration-effect relationships between plasma and brain GHB concentrations and its sedative/hypnotic effects (23). The administration of MCT inhibitors represents a novel therapeutic strategy for the treatment of GHB overdose by increasing renal clearance thereby decreasing plasma and brain concentrations leading to decreased toxicodynamic effects (14,24–26). Co-administration of GHB and L-lactate, a substrate and inhibitor of MCTs, leads to significant reductions in GHB plasma concentrations due to increased renal clearance resulting in reduced sedative/hypnotic effects and respiratory (6,14,15,24,25). MCTs are expressed throughout the body and administration of an MCT inhibitor may impact GHB transport in tissues other than the kidney. Furthermore, GHB plasma concentrations following supratherapeutic doses are at or above the in vitro K_m estimates for MCT1, 2, and 4 at pH 6.5 and 7.4, and SMCT1 (27–30), indicating that tissue distribution may become capacity-limited; however, there are no literature studies evaluating the tissue disposition of GHB. Therefore, the objective of the present study was to evaluate the role of MCTs in the tissue distribution kinetics of supratherapeutic dose of GHB, to aid in the development of therapeutic interventions for GHB overdose.

MATERIAL AND METHODS

Chemicals.

Sodium GHB was provided by the National Institute of Drug Abuse. Formic acid and sodium L-lactate were purchased from Sigma-Aldrich (St. Louis, MO). Isoflurane was purchased from Henry Schein (Melville, NY). Deuterated GHB (GHB-d6) was obtained from Cerrilliant (Round Rock, TX). Acetic acid and HPLC grade methanol, acetonitrile, and water were purchased from Honeywell Burdick and Jackson (Morristown, NJ).

Animals and Surgery.

Male Sprague-Dawley rats (Envigo, Indianapolis, IN) weighing 280 to 320 g (tissue distribution studies) or 9 weeks of age (MCT expression studies) were used. Rats were individually housed in controlled lighting (12-h light/dark cycle), temperature (20 ± 2 °C), and humidity (40–70%) with food and water provided ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Buffalo. For tissue distribution studies, rats had cannulas implanted in the right jugular vein and left femoral vein (L-lactate group only) as described previously (14,23). Surgery was performed a minimum of 2 days prior to the start of experiments. Cannulas were flushed daily with heparinized saline (40 IU/ml) to maintain patency.

GHB Tissue Distribution.

GHB naïve rats ($N = 30$ per dose) were randomly assigned to a dose group and were administered GHB intravenously (400, 600, or 800 mg/kg) or GHB (600 mg/kg iv) plus L-lactate (330 mg/kg iv bolus followed by 121 mg/kg/h infusion, 5 min after GHB administration). GHB was administered as a 200-mg/mL solution prepared in sterile water. Loss and return to righting reflex (LRR and RRR) were recorded as toxicodynamic endpoints in all animals and sleep time (RRR–LRR) was determined. Rats were exsanguinated under isoflurane anesthesia followed by collection of discrete brain regions (frontal cortex, striatum, and hippocampus), liver, kidney, spleen, heart, lung, testis, and intestine for up to 330 min post-dose ($N = 3$ per time point). Immediately following excision, tissues were blotted to remove additional residual blood, weighed, and snap frozen in liquid nitrogen. Heparinized blood samples were divided in two with one aliquot retained as whole blood and the second aliquot was separated by centrifugation at $1000 \times g$ for 10 min at 4 °C to obtain plasma. Tissue samples were stored at -80 °C until analysis, and plasma/blood samples were stored at -20 °C.

Sample Preparation.

Thawed tissue samples were homogenized on ice with double distilled water (4 ml/g tissue). A previously described anion exchange solid phase extraction protocol was utilized to extract GHB from all matrices, except discrete brain regions (23). Briefly, 5 μ L of GHB stock solution (or double distilled water for tissue samples) and 5 μ L of GHB-d6 (1 mg/mL in methanol; internal standard) were added to 50 μ L of plasma/blood or 100 μ L of tissue homogenate. For blood analysis, 100 μ L of double distilled water was added to lyse red blood cells. Acetonitrile (0.4 mL; 0.3 mL for blood) was added to precipitate proteins

followed by centrifugation at $10,000\times g$ for 20 min. Of the resulting supernatant, 0.2 mL was aspirated and combined with double distilled water (0.8 mL) followed by extraction using Bond Elut SAX cartridges (100 mg resin, 1 mL volume, Varian, Palo Alto, CA) (23). Discrete brain regions were prepared for GHB analysis as previously described (23).

LC/MS/MS Analysis.

GHB concentrations in all matrices were measured using a previously developed LC/MS/MS assay (23). Analysis was conducted on an Agilent 1100 series HPLC with an online degasser, a binary pump, and an autosampler (Agilent Technologies, Palo Alto, CA) coupled to a PE Sciex API triple-quadrupole tandem mass spectrometer with a turbo ion spray (Applied Biosystems, Foster City, CA). Mobile phase conditions and mass spectrometer parameters are detailed in Felmlee *et al.* (23).

MCT Expression.

Liver, spleen, and lung were collected from male Sprague-Dawley rats ($N=4$) following exsanguination under isoflurane anesthesia. Tissues were blotted to remove excess blood and immediately snap frozen in liquid nitrogen. Total RNA was extracted from tissues (20 mg) using an RNeasy Mini kit (Qiagen) following the manufacturer's protocol. RNA quantity/quality was assessed using a Nanodrop and stability determined using RNA Flash Gels (Lonza) to evaluate 18 and 28S rRNA. Total RNA (1000 ng) was reversed transcribed to cDNA using an RT² First Strand cDNA kit (Qiagen). Target gene products were amplified using Custom qPCR arrays (Qiagen) following the manufacturers' protocols. Gene expression was normalized using the geometric mean of 18S, β -actin, and rat plp1. Fold change for MCT1 and MCT4 expression relative to liver expression was calculated using the C_T method.

Data Analysis.

GHB concentrations in tissue were corrected for residual blood volumes using Eq. 1 (31) where $C'_{\text{GHB}(t)}$ is the measured tissue concentration, $C_{\text{GHB}(b)}$ is the blood GHB concentration, and V_F is the volume fraction of residual blood or tissue. Tissue-specific residual blood volumes were obtained from Triplett *et al.* (32); testis residual blood volume was unavailable so the residual blood volume for brain was utilized. Area under the curve ($\text{AUC}_{0-\text{inf}}$) values for plasma and tissues were calculated using the log/linear trapezoidal method with variability calculated by the Bailer method in WinNonLin version 5.2 (Pharsight Corp., Mountain View, CA) or the Bailer-Satterthwaite approximation in Phoenix version 7.0 (Certara). Time-averaged plasma and blood clearance were calculated as Dose/AUC. Tissue partitioning was calculated by the AUC method ($K_p = \text{AUC}_{\text{tissue}} / \text{AUC}_{\text{plasma}}$). Partitioning into the liver was corrected for metabolism based on in vivo Cl_{int} determined from steady-state infusions of GHB (14). Variability in tissue partitioning was calculated by the method of propagation of errors. Data was analyzed using one-way ANOVA with a Tukey's post hoc test or unpaired t tests in GraphPad Prism version 6.0 (GraphPad Inc., San Diego, CA).

$$C_{\text{GHB}(t)} = \frac{C'_{\text{GHB}(t)} - V_{\text{F(B)}} \times C_{\text{GHB(B)}}}{V_{\text{F}(t)}} \quad (1)$$

RESULTS

LC/MS/MS Assay

The lower limits of quantitation for GHB were 1 µg/ml (plasma and blood), 8 µg/g (brain), and 5 µg/g (all other tissues). The standard curve ranges were 1–500 µg/ml, 8–800 µg/g, and 5–1000 µg/g in plasma/blood, brain, and all tissues, respectively, based on regression analysis ($r^2 > 0.999$) of peak area ratios (GHB/GHB-d6) versus GHB concentration. Accuracy and precision for GHB (all matrices) were 85–105 and 0.5–1.5%, respectively.

GHB Toxicokinetics and Tissue Distribution

GHB demonstrated nonlinear plasma concentration–time profiles over the dose range studied consistent with previous IV administration studies (Fig. 1A). Similar profiles were observed for blood GHB concentrations (Fig. 1B). Blood to plasma ratios remained linear over the concentration range observed in the present study (data not shown) consistent with previous work assessing GHB partitioning into red blood cells (30). The observed GHB sedative/hypnotic effect agreed with previous studies with a significant dose-dependent increase in sleep time observed (Fig. 1C). Time-averaged plasma and blood clearance decreased with increasing dose (8.7 to 6.2 ml/min/kg, plasma; 10.9 to 8.8 ml/min/kg, blood) consistent with saturation of metabolic clearance. Morris *et al.* (14) demonstrated no diluent effect on renal and metabolic clearance with the infusion of normal saline, so rats in the dose-dependent study did not have femoral vein cannulas implanted to evaluate diluent effect. GHB tissue concentration profiles demonstrated similar terminal slopes to that observed in plasma indicating that the true elimination phase was being observed in all matrices and nonlinear distribution is not due to nonlinear binding (data not shown). AUC values were calculated for each matrix using the log/linear trapezoidal method and the Bailer method (33) and are listed in Table I. Dose-normalized tissue AUC values increased with increasing dose in all tissues except the lung, which demonstrated decreased normalized AUC values. The variability observed for the calculated AUC values was minimal suggesting that intra-individual variability in functional transporter expression is low.

GHB tissue partition coefficients for each dose were calculated using the AUC method and the results are displayed in Figs. 2 and 3. GHB partitioning varied in a tissue- and dose-dependent manner suggesting the involvement of saturable active transport processes in the distribution of GHB. Partition coefficients (K_p) did not exceed 0.5 (except in the kidney) suggesting limited uptake by diffusion or carrier-mediated mechanisms or the involvement of active efflux transporters; GHB is predominantly ionized at physiological pH, so diffusion is limited. It is also known that GHB is not protein bound in plasma so protein binding does not play a role in its tissue uptake. While K_p values were the highest in the kidney, these results are likely confounded by the presence of GHB in the tubular lumen resulting in an overestimation of K_p values. K_p values increased significantly in a dose-dependent manner

in the liver and heart, with moderate increases in the spleen suggesting saturation of efflux transporters in these tissues. This indicates that in overdose cases, GHB may accumulate in these tissues. In contrast, a dose-dependent decrease in K_p values was observed in the lung indicating saturation of active uptake transporters. Interestingly, GHB partitioning into the intestine was high following iv bolus administration suggesting that active uptake of GHB occurs at the basolateral membrane of enterocytes. Partitioning of GHB in the brain (Fig. 2) was not dose-dependent in the frontal cortex, striatum, or hippocampus with K_p values less than 0.5.

Influence of L-lactate on GHB Distribution

The impact of L-lactate on GHB disposition was evaluated in tissues that demonstrated dose-dependent changes in GHB partitioning (liver, kidney, spleen, and lung), as well as discrete brain regions (frontal cortex, striatum, and hippocampus) as the brain is the site of action. Renal clearance was not evaluated in the present study due to the destructive sampling design, which prohibited the determination of the total amount of GHB excreted in the urine, both in the presence and absence of L-lactate. In the presence of L-lactate, the plasma AUC decreased significantly consistent with previous studies (14,25), with the decrease due to inhibition of MCT/SMCT-mediated renal reabsorption of GHB and the corresponding increase in renal clearance. L-lactate is a known inhibitor of MCT1, 2, and 4, as well as SMCTs. If tissue distribution is not MCT-dependent, AUC values should decrease proportionally to the decrease in plasma AUC when GHB is co-administered with L-lactate. In the presence of L-lactate, AUC values decreased more than proportionally in the liver, striatum, and hippocampus, while kidney, lung, and spleen AUC values remained the same or increased as compared to GHB alone (Table II). Partitioning (K_p values) decreased in the presence of L-lactate in the liver and discrete brain regions (Fig. 4) with a significantly lower K_p value in the hippocampus. In contrast, increased partitioning was observed in the spleen, kidney, and lung with a significant increase in the spleen. As with GHB alone, partitioning in the kidney is likely overestimated due to the presence of GHB in the tubular lumen.

MCT Expression

MCTs can function as bidirectional transporters; however, MCT1 is thought to be primarily responsible for uptake of monocarboxylates, while MCT4 is involved in monocarboxylate efflux (17). MCT1 expression is significantly lower in the kidney, spleen, and lung (~ 3- to 4-fold lower) as compared to the liver, while the kidney, lung, and spleen demonstrated higher MCT4 expression (four- to ninefold higher) than the liver (Fig. 5).

DISCUSSION

GHB demonstrates complex toxicokinetics, including saturable renal reabsorption and oral absorption in part due to its affinity for MCTs and SMCTs. The nonlinear toxicokinetics of GHB result in high sustained systemic concentrations following the illicit use of GHB. Previous studies have demonstrated the relationship between plasma/brain GHB concentration and effect (23). Therapeutic strategies aimed at decreasing systemic exposure through inhibition of MCTs/SMCTs result in increased renal clearance (24,34) and decreased toxicodynamic effects (6,25).

Previous in vitro and in situ perfusion studies have characterized the MCT and SMCT-mediated transport of GHB to elucidate the isoforms contributing to its disposition. MCT-mediated transport of GHB has been demonstrated in the kidney (16,28), intestine (13), at the blood-brain barrier (20), and red blood cells (30). Assessment of the renal transport of GHB in rat kidney membrane vesicles (28) and HK-2 cells (16,35) showed that uptake was pH-, sodium-, and concentration-dependent consistent with the involvement of MCT1–4 and SMCT1/2. Additional studies using rMCT1 transfected cells and siRNA knockdown experiments suggested that GHB was transported by MCT1, 2, and 4, with MCT1 being the primary isoform involved in GHB transport (27,28). The affinity of GHB for these MCT isoforms varies ranging from 2 mM (MCT1; (35)) to 18 mM (MCT 2 and 4; (27)) in the presence of a pH gradient. The K_m for GHB transport via MCTs varies with pH (30) resulting in tissue-specific K_m values dependent on the pH gradient present. In the absence of a pH gradient, transport still occurs via MCTs but the apparent affinity of GHB for the transporter decreases (30), consistent with results obtained for lactate transport in red blood cells (36). SMCT1-mediated transport of GHB has been evaluated in rat thyroid FRTL cells (29) and oocytes transfected with human SMCT1 (37) with K_m values of 0.68 and 1.62 mM. The plasma GHB concentrations observed in the present study are as high as 15 mM (1560 $\mu\text{g/mL}$) indicating that saturation of MCT/SMCT isoforms may be occurring, thereby impacting the disposition of GHB. This is further complicated by the fact that MCT1 and 4 function as bidirectional transporters (38,39); however, MCT1 is thought to function primarily as an uptake transporter, while MCT4 primarily functions as an efflux transporter (39–41). Saturation of transport may occur in either direction and is likely dependent on the expression of transporters at the membrane, the specific MCT isoforms that are expressed in a given tissue and the direction of the pH gradient (driving force for transport). Intracellular pH is typically 7.0 to 7.3 but can be as low as pH 6.0 with increased acid production which has been observed in muscle (42). An outward pH gradient suggests that efflux may be the predominant MCT-mediated pathway determining the tissue distribution of GHB, which is consistent with a K_p less than 1 as observed in the present study.

MCT1 is ubiquitous in rats and humans and inhibition of renal MCTs alters renal clearance in both species (25,43). Inhibiting MCT1 or its related isoforms MCT2 and MCT4 may alter the tissue distribution of GHB which has been demonstrated to be a substrate for all three MCT isoforms. While SMCT distribution is more restricted, expression is observed in the human and rat kidney (22), and inhibition may impact the renal clearance of GHB. MCT1 is the only isoform expressed at the rat and human blood-brain barrier (luminal and abluminal expression in rats) and, therefore, is the sole determinant of GHB distribution into the brain (44). The present study represents the first evaluation of the tissue distribution of GHB, demonstrating complex nonlinear tissue distribution kinetics which occurs in a dose- and tissue-dependent manner. Further, there is the potential to observe nonlinear GHB tissue distribution in humans, as K_m values for MCTs are consistent between rats and humans. GHB accumulates in the liver, spleen, and heart with increasing dose suggesting saturation of active efflux. In contrast, partitioning into the lung decreases with dose suggesting saturation of an active influx transporter, which is consistent with the lower level of MCT1 expression in this tissue. Inhibition by L-lactate resulted in partitioning changes in the striatum, hippocampus, liver, kidney, spleen, and lung, confirming the role of MCTs in GHB

distribution and supporting tissue-specific distribution kinetics. Distribution changes observed with increasing GHB dose and L-lactate administration were consistent with tissue differences in MCT1 and MCT4 mRNA expression. Partitioning increased in the kidney, spleen, and lung, which are all tissue with higher MCT4 expression. In contrast, partitioning in the liver, where MCT1 has greater expression, increased with L-lactate administration. This is consistent with changes observed in the striatum and hippocampus, where blood-brain barrier expression of MCT1 determines partitioning (44). The lower K_p values observed at low doses in the liver and heart may be related to both differences MCT1 and MCT4 expression levels and alterations in the pH gradient due to the presence of higher concentrations of GHB and L-lactate. Higher intracellular concentrations of L-lactate and GHB, both monocarboxylic acids, may lead to decreased intracellular pH. A lower intracellular pH would result in a shift from an inward to an outward pH gradient leading to changes in the directionality of GHB transport. In the kidney, changes in tubular lumen pH effect the renal clearance of GHB; increasing renal pH through the administration of sodium bicarbonate leads to a decrease in renal reabsorption clearance with a corresponding increase in total renal clearance consistent with reduce transport by renal MCTs. The observed complexities and tissue-dependent partitioning are likely due to the bidirectional function of the MCT family of transporters (38,39) and the tissue-specific involvement of one or more of these transporters.

GHB demonstrates negligible plasma protein binding; therefore, if GHB underwent unrestricted diffusion, we would expect equal plasma and tissue concentrations with K_p values of 1. However, GHB is ionized at physiological pH and therefore exhibits limited diffusion into tissue. The low K_p values observed in the present study may be a result of limited diffusion into tissues or active uptake and efflux. Methods to distinguish between diffusion and active uptake or efflux include examining the concentration dependence in the tissue AUC values or altering MCT-mediated transport either via inhibition, gene knockdown, or overexpression. In the present study, dose-dependent partitioning was observed in the liver, spleen, lung, and heart. Co-administration of L-lactate, a known inhibitor of multiple MCT isoforms, altered GHB tissue distribution in discrete brain regions, liver, spleen, and lung. In our previous study, co-administration of GHB (600 mg/kg iv) and L-lactate resulted in decreased partitioning of GHB into the brain extracellular fluid (21) further supporting the role of MCT1 in GHB distribution, as MCT1 is the sole isoform expressed on the blood-brain barrier.

While MCT1 is ubiquitous, the tissue distribution and subcellular localization of the other isoforms are quite different, and this may contribute to the observed differences in tissue disposition kinetics. Based on our in vivo results, a single transporter functioning bidirectionally or two transporters (one uptake, one efflux) may be responsible for GHB distribution into the liver, kidney, spleen, lung, and heart. Inhibition of MCTs by lactate resulted in decreased partitioning in the liver and increased partitioning in the kidney, lung, and spleen, supporting the involvement of both active uptake and efflux by MCTs in GHB tissue distribution. Quantitative mRNA expression in the liver, kidney, spleen, and lung suggests that variations in MCT1 and MCT4 expression levels in these tissues may contribute to the observed complexities in GHB tissue distribution. MCT1 expression is higher in the liver, consistent with inhibition of uptake leading to decreased K_p values in the

presence of lactate. K_p values increased in the kidney, lung, and spleen corresponding to the higher MCT4 (efflux transporter) expression in these tissues. Alterations in the direction of the pH gradient due to intracellular accumulation of L-lactate (decreased intracellular pH resulting in an outward pH gradient) may have contributed to the observed GHB partitioning changes observed in the presence of L-lactate. A switch to an outward pH gradient would lead to both MCT1 and MCT4 functioning as efflux transporters. Additional quantitative mRNA and protein expression data for MCT and SMCT tissue distribution and tissue-specific changes in pH gradients with L-lactate administration are not available. Such information may be crucial to further elucidate the specific pathways contributing to the transporter-mediated distribution of GHB.

An additional consideration is the potential for interindividual variability in MCT/SMCT expression. The present study addressed distribution in a rat model with minimal variability in transporter expression; alterations in MCT/SMCT expression may impact overall drug distribution and toxicokinetics. There is little information in the literature regarding the regulation of MCTs with the majority of studies focusing on MCT1. MCT1 and MCT2 expression in the brain were increased in rats fed a high fat diet and genetically obese rats suggesting that obesity could contribute to upregulation of MCTs (45). In addition, induction of MCT1 expression has been observed with GHB and butyrate exposure (46,47) indicating that chronic exposure to MCT substrates may lead to increased expression levels. Few studies are available assessing the impact of polymorphic variants of MCTs/SMCTs on transporter function; recent studies have identified potential functional MCT polymorphisms that influence plasma lactate accumulation (48) and prognosis in colorectal cancer (49). Future studies should assess the impact of altered MCT expression on the distribution and toxicokinetics of GHB.

A further complexity in the present study is the lack of quantitative information regarding the tissue-specific metabolism of GHB. GHB metabolism has been demonstrated to occur in the brain cells via aldo-keto reductase 1A1 and 7A2 (AKR1A1 and AKR7A2), aldehyde dehydrogenase 5A1 (ADH5A1), and alcohol dehydrogenase, iron-dependent 1 (ADHFe1) (50). Expression of these enzymes has been demonstrated in the brain, liver, and kidney (50–52). Enzyme kinetic estimates for AKR1A1-mediated GHB metabolism have been evaluated in hepatocytes and suggest that AKR1A1 has a low affinity and high capacity for GHB metabolism (53). Partitioning into metabolic tissues must be corrected for the intrinsic clearance of a drug within the tissue. In the present study, metabolic clearance was saturated and the predicted K_m value based on previous studies (14,15) is well below all of the measured plasma concentrations. Based on L-lactate/GHB interaction studies, GHB metabolism is not altered in the presence of L-lactate (25). Partitioning in the liver was corrected for metabolism (based on previous in vivo infusion studies (14)), as this is likely the primary metabolic site following supratherapeutic doses. However, K_p values in other tissues were not corrected for metabolism due to the paucity of information on GHB tissue metabolism.

CONCLUSION

In summary, we have demonstrated that GHB distribution occurs in a dose- and tissue-dependent manner following the administration of supratherapeutic doses consistent with the involvement of the MCT family of transporters. Further quantitative understanding of tissue-specific expression and transport kinetics for MCT-mediated influx and efflux is required. Additional studies are needed to comprehensively evaluate the effects of L-lactate (with respect to L-lactate dose and administration time) and the efficacy of more potent and specific MCT inhibitors, as well as the influence of variability in MCT expression on tissue distribution of GHB to better predict the impact of MCT inhibition on GHB toxicokinetics and toxicodynamics.

ACKNOWLEDGEMENTS

The study received grant support from the NIH (R01 DA023223).

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Fig. 1.

Plasma (a) and blood (b) concentration–time profiles following administration of 400 mg/kg (dotted line), 600 mg/kg (dashed line), or 800 mg/kg (solid line) GHB intravenously ($N = 3$ per time point). Data are presented as mean $\pm$ SD. c Sleep time (return minus loss of righting reflex) following 400, 600, or 800 mg/kg GHB intravenously. Data are presented as mean $\pm$ SD, $N = 17$ –19. * $P < 0.05$ when compared to 400 mg/kg. † $P < 0.05$ when compared to 600 mg/kg

Fig. 2. GHB partition coefficients (K_p) in discrete brain regions for rats administered 400, 600, or 800 mg/kg intravenous GHB ($N = 30$ per dose). K_p values were determined by the AUC method using the data presented in Table I. Data are presented as mean $\pm$ SEM

Fig. 3. GHB partition coefficients (K_p) in selected tissues for rats administered 400, 600, or 800 mg/kg GHB intravenously ($N = 30$ per dose). K_p values were determined by the AUC method using the data presented in Table I. Data are presented as mean $\pm$ SEM. * $P < 0.05$ when compared to 400 mg/kg. † $P < 0.05$ when compared to 600 mg/kg

Fig. 4.

GHB partition coefficients (K_p) in selected tissues (a) and discrete brain regions (b) for rats administered 600 mg/kg GHB intravenously (alone) or with L-lactate (330 mg/kg iv followed by 121 mg/kg/h iv infusion) ($N= 24$). K_p values were determined by the AUC method using the data presented in Table II. Data are presented as mean $\pm$ SEM. * $P < 0.05$ when compared to 600 mg/kg GHB alone

Fig. 5. MCT1 (a) and MCT4 (b) mRNA expression in the liver, kidney, spleen, and lung of 9-week-old male Sprague-Dawley rats. Transporter expression was quantified using Custom RT² Profiler Arrays and expression relative to liver was normalized using the arithmetic mean of three housekeeping genes (18S, β -actin, and ribosomal protein large P1). Mean $\pm$ SD, $N=3-4$ /sex. * $P < 0.05$ when compared to liver

Table 1.

AUC and Dose-Normalized AUC Values Following the Administration of 400, 600, or 800 mg/kg GHB Intravenously ($N = 30$ Per Dose)

GHB dose (mg/kg)	AUC (min mg/mL)			Dose-normalized AUC		
	400	600	800	400	600	800
Plasma	45.9 ± 5.45	86.5 ± 4.26	129 ± 6.39	0.11	0.14	0.16
Brain						
Frontal cortex	11.0 ± 0.99	18.5 ± 1.03	38.9 ± 3.41	0.027	0.031	0.049
Striatum	9.51 ± 0.88	16.3 ± 0.64	29.6 ± 2.05	0.024	0.027	0.037
Hippocampus	8.07 ± 1.24	18.6 ± 0.69	27.9 ± 1.65	0.020	0.031	0.035
Tissues						
Liver	1.87 ± 0.65	11.4 ± 1.35	26.4 ± 2.35	0.0046	0.019	0.033
Kidney	28.3 ± 3.43	105 ± 14.4	127 ± 6.73	0.071	0.175	0.158
Spleen	9.35 ± 0.97	18.1 ± 1.54	34.9 ± 1.59	0.023	0.030	0.044
Lung	19.8 ± 1.73	27.7 ± 1.11	32.6 ± 1.72	0.050	0.046	0.041
Heart	6.66 ± 0.67	25.1 ± 1.45	37.3 ± 2.35	0.017	0.042	0.046
Intestine	18.2 ± 1.83	35.1 ± 1.60	54.4 ± 1.63	0.045	0.058	0.068
Testis	13.8 ± 1.17	31.6 ± 2.91	42.2 ± 1.66	0.034	0.053	0.052

Tissue concentrations were corrected for residual blood content using Eq. 1. AUC values were calculated using the log/linear trapezoidal method and variability was calculated using the Bailer method. Data are presented as mean ± SEM

AUC area under the curve, GHB γ -hydroxybutyric acid

Table II.

AUC Values (min mg/ml) Following the Administration of 600 mg/kg GHB Intravenously (Alone) or with L-lactate (330 mg/kg IV Followed by 121-mg/kg/h IV Infusion) (*N* = 24)

		AUC	
		GHB	GHB + lactate
Brain	Plasma	84.26 ± 4.62	66.9 ± 1.11
	Frontal cortex	18.86 ± 1.02	15.56 ± 0.41
	Striatum	16.20 ± 0.63	12.54 ± 0.46
	Hippocampus	18.25 ± 0.69	11.00 ± 0.37
Tissues	Liver	11.60 ± 1.35	7.47 ± 0.54
	Kidney	105 ± 14.4	105 ± 3.39
	Spleen	18.83 ± 1.54	22.24 ± 0.87
	Lung	28.37 ± 1.11	28.13 ± 1.03

Tissue concentrations were corrected for residual blood content using Eq. 1. AUC values (min mg/mL) were calculated using the log/linear trapezoidal method and variability was calculated using the Bailer-Satterthwaite approximation. Data are presented as mean ± standard error of the mean