DMD Fast Forward. Published on August 21, 2008 as doi:10.1124/dmd.108.022996

Effects of L-lactate and D-mannitol on γ-Hydroxybutyrate Toxicokinetics and Toxicodynamics in Rats

Qi Wang[1], Xiaodong Wang[1] and Marilyn E. Morris

Department of Pharmaceutical Sciences

School of Pharmacy and Pharmaceutical Sciences

University at Buffalo, State University of New York

Amherst, New York 14260, USA

a) Running Title: GHB Toxicokinetics and Toxicodynamics

b) Corresponding author: Marilyn E. Morris, Ph.D.

517 Hochstetter Hall

Department of Pharmaceutical Sciences

School of Pharmacy and Pharmaceutical Sciences

University at Buffalo

State University of New York

Amherst, New York 14260

USA

Phone: 1 (716) 645-2842 ext. 230

Fax: 1 (716) 645-3693

E-mail: memorris@buffalo.edu

c) Document statistics:

number of text pages… 32

number of tables… 3

number of figures…4

number of references… 33

number of words in the Abstract… 245

number of words in the Introduction… 755

number of words in the Discussion…

d) Abbreviations: GHB, γ-hydroxybutyrate; GABA, γ-aminobutyric acid; LC/MS/MS, liquid chromatography-tandem mass spectrometry; MCT, monocarboxylate transporter;

Abstract

Overdoses of γ-hydroxybutyrate (GHB), a drug of abuse, result in coma, respiratory arrest, and death. The objective of this study was to evaluate a potential GHB detoxification strategy by inhibiting the monocarboxylate transporter (MCT)-mediated renal reabsorption of GHB in rats, using the MCT substrate L-lactate. The use of the osmotic diuretic D-mannitol alone or combined with L-lactate was also explored. GHB (208 $mg \bullet hr^{-1}kg^{-1}$) was infused *iv* for 3 hours in the absence or presence of L-lactate (60.5, 121, and 302.5 $mg \bullet hr^{-1}kg^{-1}$), D-mannitol (0.5 g/kg), or L-lactate (60.5 $mg \bullet hr^{-1}kg^{-1}$) combined with D-mannitol (0.5 g/kg). GHB in plasma and urine samples was determined along with blood pH, electrolytes, glucose, and L-lactate. Administration of L-lactate, or the combination of L-lactate and D-mannitol, but not D-mannitol alone, significantly increased the renal and total clearances of GHB in rats. Blood pH and electrolyte concentrations exhibited small changes with GHB, GHB/lactate and GHB/mannitol treatments, although most values remained within their normal range. The concomitant administration of Lactated Ringer's solution (28 mM L-lactate) at 300 μl/min with mannitol (0.5 g/kg) resulted in a significant increase in GHB clearance and a decrease in sleep time following an iv dose of 1 g/kg. Overall, our results indicated that 1) the use of the MCT inhibitor L-lactate can increase the renal and total clearances of GHB, and 2) the combination of Lactated Ringer's solution and D-mannitol significantly alters GHB toxicokinetics and toxicodynamics, and represents a potential clinical detoxification strategy for the treatment of GHB overdoses.

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

The presence of γ-hydroxybutyrate (GHB) has been demonstrated in the mammalian brain, where it is primarily formed from γ-aminobutyric acid (Fishbein and Bessman, 1964). Endogenous GHB has also been detected in various tissues other than the brain, including heart, kidney, liver, lung, muscle and gastrointestinal (GI) tract (Nelson et al., 1981; Tedeschi et al., 2003). GHB has been proposed to be a neurotransmitter or neuromodulator with its own specific receptor identified in the brain (Andriamampandry et al., 2003). Clinically, GHB has been used to treat alcohol and heroin dependence (Gallimberti et al., 2000) and sleep disorders in Europe (Mamelak et al., 1986); it has been approved in the United States for the treatment of cataplexy attacks and to reduce excessive daytime sleepiness in patients with narcolepsy. However, GHB has been extensively used as a popular steroid alternative by body builders, as a recreational drug at nightclubs and rave parties, and as a means of drug-facilitated sexual assaults (Schwartz et al., 2000; Okun et al., 2001). Serious adverse effects have been associated with GHB overdoses including coma, seizure, and even death (Mason and Kerns, 2002). GHB or its prodrugs, γ-butyrolactone and 1, 4-butanediol, are especially toxic when mixed with alcohol and/or other recreational drugs to increase its euphoric effects, and this has led to significant morbidity and mortality (Okun et al., 2001). There were over 7100 reports of GHB overdoses and 65 GHB related deaths in the US from 1990-2000 (Shannon and Quang, 2000). The treatment of GHB overdoses consists mainly of supportive care, and no specific antidotes have been reported for clinical use (Mason and Kerns, 2002).

The physiological, pharmacological and toxicological effects of GHB are believed to be mediated by its own receptor and the γ-aminobutyric acid B ($GABA_B$) receptor (Maitre, 1997).

Although contradictory reports exist in the literature, there is increasing evidence suggesting that the hypnotic effect of GHB is mediated by the $GABA_B$ receptor by two putative pathways: directly acting on the $GABA_B$ receptor as a partial agonist (Carai et al., 2001); and indirectly acting on $GABA_B$ receptor by inter-converting GHB to GABA (Hechler et al., 1997). The affinity of GHB for the $GABA_B$ receptor is very low, and the IC_{50} value for GHB displacing GABA is above 100μM (Bernasconi et al., 1992). It is believed that GABA converted from exogenous GHB is sufficient to induce the hypnotic effects associated with GHB (Hechler et al., 1997).

Nonlinear pharmacokinetics of GHB has been reported for rats (Lettieri and Fung, 1979) and humans (Ferrara et al., 1992), and the mechanisms underlying the nonlinear pharmacokinetics of GHB include capacity-limited metabolism (Lettieri and Fung, 1979; Ferrara et al., 1992), capacity-limited absorption (Arena and Fung, 1980), and capacity-limited reabsorption in the proximal tubules mediated by pH-dependent and sodium-dependent monocarboxylate transporters (Morris et al., 2005; Wang et al., 2006). Since the metabolic clearance is saturated following high doses of GHB, the renal clearance represents a significant route of elimination at these doses and the renal clearance of GHB can be increased by inhibiting the renal reabsorption of GHB (Morris et al., 2005). L-lactate is a typical substrate of MCT1-4 (Halestrap and Price, 1999), and can significantly inhibit the uptake of GHB in membrane vesicles isolated from rat proximal tubule cells (Wang et al., 2006). We have previously reported that a high dose of L-lactate can reduce the renal reabsorption of GHB in rats in vivo (Morris et al., 2005). In that same study, we also reported that a high dose of the osmotic diuretic D-mannitol is effective in increasing GHB renal clearance (Morris et al., 2005). However, the effects of more clinically

relevant lower doses of L-lactate and mannitol, and that of the combination of L-lactate and mannitol, on the toxicokinetics (TK) of GHB have not been examined. As well, studies have not previously examined the effects of these treatments on the toxicodynamics (TD) of GHB. Our hypothesis is that increasing the renal clearance of GHB following overdoses will increase its overall clearance and decrease its toxicity, representing a potential strategy to treat overdoses. The objectives of this study were (1) to characterize the dose-dependent effects of L-lactate, D-mannitol and the combined treatment of L-lactate and D-mannitol on the steady-state TK of GHB, and (2) to determine the effects of Lactated Ringer's solution and mannitol on the TK and TD (hypnotic effect) of GHB after a single iv bolus dose of GHB (1 g/kg). L-lactate was administered as Lactated Ringer's solution in order to evaluate the potential use of this clinically available and widely-used form of L-lactate.

Material and Methods

Chemicals and reagents

GHB (as sodium salt), L-(+)-lactate (as sodium salt), formic acid, anthrone, inulin and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). The internal standard sodium GHB-D6 (1 mg/ml) was purchased from Cerilliant Corp. (Round Rock, TX). Lactated Ringer's solution was purchased from Henry Schein (Melville, NY). Methanol and HPLC grade water were purchased from Fisher (Fairlawn, NJ). Concentrated H_2SO_4 (93%) was purchased from JT Baker (Phillipsburg, NJ).

Animals and surgery

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used as our animal model. The body weights of the rats were 240-280g and ~200g for the infusion study with L-lactate and the infusion study using Lactated Ringer's solution, respectively. The rats were randomly assigned to study groups and kept in individual cages after surgery. The animal housing room had controlled environmental conditions with temperature and relative humidity of approximately 20 ± 2°C and 40-70%, respectively, and artificial lighting, alternating on a 12-hour light-dark cycle. All care and experiments were approved by the Institutional Animal Care and Use Committee at the University at Buffalo. The rats had cannulas implanted as previously described (Morris et al., 2005). Briefly, rat right jugular veins, left femoral veins and bladders were cannulated under anesthesia following an intramuscular injection of ketamine 90 mg/kg and xylazine 9 mg/kg (Henry Schein, Melville, NY). The animals were allowed 3 to 4 days for recovery from surgery before the experiment.

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

Infusion studies and sample collection

The rats were placed in metabolism cages for collection of urine during the study period. GHB, inulin, L-lactate, and/or D-mannitol dissolved in sterile water were administered via an intravenous (i*v*) bolus injection followed by an *iv* infusion through the femoral veins to rats ($n \geq 3$ each group). Before the infusion, the solutions were sterilized by passing them through 0.2 μm filters. All the infusion solutions were prepared to maintain a total osmolarity of 320 mOsm, and pH of 7.4. The components of the infusion solution for GHB only (control) were sodium GHB (159 mM) and inulin (1.9 mM). The components of the infusion solutions for L-lactate treatments were sodium GHB (121, 97, and 62 mM for L-lactate doses of 60.5, 121 and 302.5 $mg \bullet hr^{-1}kg^{-1}$, respectively), sodium L-lactate (40, 64, and 102 mM for L-lactate doses of 60.5, 121 and 302.5 $mg \bullet hr^{-1}kg^{-1}$, respectively), and inulin (1.5, 1.2 and 0.8 mM for L-lactate dose of 60.5, 121 and 302.5 $mg \bullet hr^{-1}kg^{-1}$, respectively). The infusion rate was different for different doses of L-lactate in order to use iso-osmotic infusion solutions. Blood samples for the measurement of GHB (100 μl) were withdrawn from the jugular vein at different time points and placed in heparinized 0.6-ml microcentrifuge tubes. For the measurement of blood pH and electrolytes, the blood samples (300 μl each) were withdrawn into 1 ml heparinized syringes and stored on ice (< 30 min) before analysis (ABL 605 Radiometer, Copenhagen, Denmark). The plasma was separated from whole blood by centrifugation at $2{,}000 \times g$ for 5 min at 4°C. For urine collection at 1 or 3 hr, the bladder was rinsed with warm normal saline (2 ml) at the end of each collection. Urine pH and volume were measured right after the collection. For the brain samples, the animals were sacrificed at the end of the 3-hour infusion period, and whole brains were harvested for drug concentration analysis. All plasma, urine and brain samples were stored

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

at -80°C until analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS) (Fung et al., 2004) to determine GHB concentrations.

Experimental design

Effects of L-lactate and/or mannitol on the steady-state TK of GHB

Based on our previous study (Morris et al., 2005), a single dose of GHB, 400 mg/kg *iv* bolus followed by 208 $mg \bullet hr^{-1}kg^{-1}$ was selected to represent a high dose of GHB. Three different doses of L-lactate were used in this study: 130 mg/kg *iv* bolus followed by 60.5 $mg \bullet hr^{-1}kg^{-1}$, 330 mg/kg *iv* bolus followed by 121 $mg \bullet hr^{-1}kg^{-1}$, and 330 mg/kg *iv* bolus followed by 302.5 $mg \bullet hr^{-1}kg^{-1}$ to achieve three steady-state L-lactate blood concentration elevation of ~0.1, ~0.2 and ~0.4 mM, respectively. An *iv* dose of 0.5 g/kg of D-mannitol was used to study the effect of osmotic diuresis. A combined dosing regimen of 0.5 g/kg of D-mannitol by *iv* bolus and L-lactate 60.5 $mg \bullet hr^{-1}kg^{-1}$ by *iv* infusion was used to determine the effect of combining the two treatments. The glomerular filtration rate (GFR) was determined from the clearance of inulin, as previously described (Morris et al., 2005). The 60 mg/kg *iv* bolus dose of inulin, followed by a 100 $mg \bullet hr^{-1}kg^{-1}$ *iv* infusion, was used in these studies (Darling and Morris, 1991). Blood samples were collected at 0 (blank), 10, 20, 30, 60, 90, 120 and 180 min, and urine samples at 0 (blank) and between 1 and 3 h.

Effect of Lactated Ringer's solution and/or Mannitol on GHB TK and TD

For Lactated Ringer's solution infusion studies, a single dose of GHB (1000 mg/kg *iv* bolus) was given to all rats (control, D-mannitol alone, Lactated Ringer's solution alone, and D-mannitol/Lactated Ringer's solution combination group). In the control group, saline was

infused at the rate of 300 μl/min, starting immediately after the *iv* bolus of GHB. In the D-mannitol treatment group, an *iv* bolus dose of 0.5 g/kg of D-mannitol followed by an *iv* infusion of saline, administered immediately after the *iv* bolus of GHB, was used to study the effect of osmotic diuresis on GHB disposition. In the Lactated Ringer's solution treatment group, Lactated Ringer's solution was infused at the rate of 300 μl/min immediately following the *iv* bolus of GHB and a loading dose of sodium L-lactate (0.4 g/kg). In the combined dosing regimen group, a dose of 0.5 g/kg of D-mannitol by *iv* bolus and Lactated Ringer's solution by *iv* infusion was administered to rats immediately after the *iv* bolus of GHB. Blood samples were collected at 0 (blank), 5, 15, 30, 60, 120, 180, and 240 min, and urine samples at 0 (blank) and between 2 and 4 h. The hypnotic effect of GHB following various treatments was determined by the sleep time, which was measured as the difference between the time of loss of the righting reflex (LRR) and regaining of the righting reflex (RRR). LRR and RRR are the indicators for the onset and offset of sleep induced by GHB, respectively.

Determination of TK parameters

The total clearance of GHB (CL) was determined from its plasma concentration at steady-state (C_{ss}) and infusion rate (k_0), using the equation k_0/C_{ss}. In the GHB *iv* bolus study, CL was determined from Dose/AUC, where AUC is the area under the plasma concentration-time curve. Renal clearance (CL_R) was determined by dividing the urinary excretion rate (dA_e/dt) by the mean plasma concentration of GHB (C_{ss}) or $A_{e,\infty}/AUC$, where A_e is the amount of GHB excreted in the urine and $A_{e,\infty}$ is the amount of GHB excreted in the urine from time 0 to ∞. The fraction of the dose eliminated by renal excretion (f_e) was determined by CL_R/ CL or $A_{e,\infty}$/Dose. GFR was determined from the inulin clearance ($k_{0,IN}/C_{ss,IN}$), where $k_{0,IN}$ and $C_{ss,IN}$ represent the

infusion rate and plasma concentration at steady-state of inulin. The renal filtration rate of GHB was determined as the product of GFR and the unbound (free) plasma concentration (C_u), where C_{ss} is approximately equal to C_u since GHB is negligibly protein bound in rat plasma (Morris et al., 2005). The renal tubular reabsorption rate of GHB was calculated by the difference between renal filtration rate and urinary excretion rate, assuming there is negligible renal secretion of GHB. A lack of renal secretion has been reported for the ketone bodies of β-hydroxybutyrate (the congener of GHB with the hydroxyl group at C-3) and acetoacetate, even at high plasma concentrations of 15 mM (Ferrier et al., 1992). Since both GHB and inulin reached steady-state by 1 hour, the average steady-state concentrations for 1-3 hour were used for C_{ss} and C_{mid} in the calculations of GFR, CL_R and CL. The metabolic clearance (CL_m) could be calculated indirectly from our data using the equation $CL_m = CL - CL_R$, assuming that total plasma clearance of GHB is equal to renal clearance plus metabolic clearance.

LC/MS/MS Assay

GHB in rat plasma, urine and brain samples were measured by a validated LC/MS/MS assay as previously described (Fung et al., 2004), with some modifications. Briefly, the LC/MS/MS system consisted of a PE SCIEX API 3000 triple-quadruple tandem mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a turbo ion spray (PE SCIEX), a Series 200 PE micro pump (Perkin-Elmer, Shelton, CT), and a Series 200 PE autosampler (Perkin-Elmer). The separation by reversed-phase high-performance liquid chromatography was performed using an Aqua C-18 5 μm 125Å column (150 × 4.6 mm i.d., Phenomenex, Torrance, CA) protected by a C-18 5 μm guard column cartridge system (Phenomenex, Torrance, CA). Compounds were eluted isocratically with a mobile phase consisting of 5 mM formic acid/methanol (33:67, v/v).

The flow rate was 0.75 ml/min and the injection volume was 10 μl (the flow rate was achieved by a splitter, so the actual flow to mass-spectrum was 0.25 ml/min and injection volume was 3.3 μl). The mass spectrometer was operated in a positive ionization mode with an optimized ion-yield setting for GHB. The interface temperature was set at 400°C. The declustering potential and collision energy for fragmentation were set at 30 and 13 eV, respectively. Multiple reaction monitoring was used for specific detection of GHB and GHB-D6 by MS/MS measuring the ion pair transitions of *m/z* 105 (parent ion) to *m/*z 87 (product ion) and of *m/z* 111 (parent ion) to *m/z* 93 (product ion), respectively. The retention time of GHB and GHB-D6 was 2.6 min and there were no interfering peaks in plasma, brain and urine samples. The Analyst software version 1.4.1 (PE SCIEX) was used for instrument control and data analysis.

Plasma, brain and urine sample preparation and assay

To each blank plasma, brain or urine sample (50 μl; appropriately diluted with H_2O), GHB-D6 stock solution (6 mM, 5 μl) and varying concentrations of GHB stock solution were added in order to prepare standards of GHB (60, 240, 600, 1200, 2400, 4800, and 7200 μM as final concentrations) for the calibration curve. The internal standard GHB-D6 was added in the same concentration and volume to each plasma, brain or urine sample. The proteins present in plasma, brain and urine were precipitated using 50% methanol (1 volume of 100% methanol to 1 volume sample). After centrifugation at 22,000 g for 20 min, the supernatant was collected for the LC/MS/MS assay. Quality control samples at low (240 μM), medium (1200 μM) and high (4800 μM) concentrations of GHB were prepared by the same procedures. The linearity of calibration curve was evaluated by regression analysis of peak height ratios (GHB/GHB-D6) to GHB concentrations in blank plasma and urine samples, respectively. The accuracy was determined

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

by comparing the calculated concentration using calibration curves of known concentrations. The precision was determined by the coefficient of variation (CV %). Within-day variability was assessed through the analysis of quality controls in triplicate, and between-day variability was determined through the analysis of quality controls on four to five separate days.

Inulin Assay

The inulin concentrations in plasma and urine samples were determined by a colorimetric method based on the procedure of Davidson and Sackner (Davidson and Sackner, 1963), as previously described (Morris et al., 2005). Briefly, inulin (10μl) was added to blank plasma (10 μl) or urine sample (10 μl; diluted with H_2O) to prepare a series of standards of inulin (5, 10, 20, 40, 60, 80 and 100 μg/ml as final concentrations) for the calibration curve. The protein content of each plasma (10 μl) or urine (10 μl; appropriately diluted with H_2O) sample was precipitated by incubating with 40 μl of trichloroacetic acid (1.0 N) for 30 min. After centrifugation at 11,000 × *g* for 5 min, the supernatant (20 μl) was mixed with 120 μl of anthrone reagent (0.08%, w/v) that was prepared by dissolving anthrone in H_2O/H_2SO_4 (10:53, v/v). The mixture was vortexed for 5 seconds, chilled on ice for 1 min, and incubated at 38°C in a water bath for 50 min. At the end of the incubation, the mixture was chilled on ice for 2 min and allowed to stand at room temperature for 20 min to equilibrate. The mixture (100 μl) was transferred to a 96-well microplate and the absorbance was determined by microplate spectrometer Spectra Max 340PC (Device Molecular, Sunnyvale, CA) at 623 nm at 25°C. The color was stable for as long as 3 hour. The assay was validated for sensitivity, linearity, accuracy and precision based on intra-plate and inter-plate analysis.

Blood ion and metabolite measurements

The measurements of ion and metabolite concentrations in rat venous blood were conducted using a radiometer (ABL 605, Copenhagen Denmark), which was equipped with multiple electrodes for measurement of Na^+, K^+, Ca^{2+}, Cl^-, pH, pCO_2, pO_2, glucose, and L-lactate. Venous blood samples, withdrawn from the right jugular vein at 0 and 3 hours were analyzed using the ABL 605 radiometer. The blood samples were stored on ice right after sampling and the samples were analyzed within 30 min.

Statistical Analysis

Statistical comparisons among more than two treatments employed one-way ANOVA (Prism 3.0 software, GraphPad, San Diego, CA) followed by a Dunnett's post-hoc test. A paired Student's t-test was used for comparing two treatments. Differences were considered to be significant when $p < 0.05$.

Results

LC/MS/MS assay for GHB in plasma, brain, and urine

The lower limit of quantitation (LLOQ) was determined to be 60 μM. The endogenous concentrations of GHB in blood, brain and urine are in the range of 10 - 40 μM as determined previously (Nelson et al., 1981; Fung et al., 2004), and the blood, urine, and brain concentrations of GHB after GHB administration were in the range of 1 - 20 mM. Therefore, the endogenous concentration of GHB was negligible in samples and omitted in the calculation of GHB concentration. The calibration curve for plasma was linear from 60 to 7200 μM based on the regression analysis ($r^2 > 0.99$) of peak height ratios (GHB/GHB-D6) versus GHB concentrations. The accuracy and precision (CV %) for plasma samples was 96-112 % and 2.4-12.7 %, respectively. The calibration curve for urine was linear from 240 to 4800 μM based on the regression analysis ($r^2 > 0.992$) of peak height ratios (GHB/GHB-D6) versus GHB concentrations. The accuracy and precision (CV %) for urine samples was 95-119 % and 3.0-8.7 %, respectively. The calibration curve for brain was linear from 60 to 1200 μM based on the regression analysis ($r^2 > 0.998$) of peak height ratios (GHB/GHB-D6) versus GHB concentrations. The accuracy and precision (CV %) for brain samples was 93-111 % and 3.4-7.6 %, respectively.

Dose-dependent effects of L-lactate on GHB TK

Our studies demonstrated that the plasma concentrations of GHB reach an apparent steady-state after 1 hour of administration. The steady-state plasma concentrations of L-lactate determined at the end of a 3 hour-infusion were dose-dependent, and increased with the L-lactate doses (Table 1). The plasma concentration of GHB was significantly decreased after the administration of L-

lactate, and the lowest concentration achieved was 0.38 mg/ml at a dose of L-lactate of 302.5 mg/ml. The steady-state renal clearances of GHB were significantly increased after the administration of L-lactate at all three doses (Fig 1), and the highest renal clearance of GHB occurred following the infusion of L-lactate 302.5 $mg \bullet hr^{-1}kg^{-1}$. The total clearance of GHB was also significantly increased after the administration of L-lactate, with the highest values observed following the administration of L-lactate 302.5 $mg \bullet hr^{-1}kg^{-1}$ (Fig 1). However, the metabolic clearance values, which were calculated as total clearance minus renal clearance, were not significantly different from that of the control (Fig 1). The GFR values were not significantly changed after L-lactate dosing (Table 1). The amount of GHB reabsorbed in the kidney was significantly decreased after L-lactate dosing in a dose-dependent manner (Table 1).

The total brain concentrations of GHB after a 3-hour infusion were determined and expressed as mg/g tissue (Table 1). Since the plasma concentration of GHB reached steady-state by 1 hour, it is likely that the 3-hour brain concentration also represents a steady-state concentration. There were no significant differences among the brain concentrations of control and L-lactate-treated groups.

pH and electrolyte concentrations in blood after GHB and L-lactate treatments

The blood pH of animals receiving GHB treatment was not significantly changed after a 3-hour infusion; however, the blood pH was increased after a 3-hour infusion of GHB with each of the three doses of L-lactate. The greatest pH change was observed with the highest L-lactate dose, with the mean pH values changing from 7.46 to 7.53 (Table 2). The concentrations of Na^+, K^+, and Ca^{2+} were not significantly changed after a 3-hour infusion of GHB in both control and

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

treatment groups (Table 2). The Cl^- concentrations were slightly decreased after a 3-hour GHB infusion in all groups, but the concentrations were still within the normal range (Table 2). Anion gaps were significantly increased with the infusion of GHB alone and with the infusion of GHB/L-lactate 302.5 $mg \bullet hr^{-1}kg^{-1}$ (Table 2). Anion gap refers to the difference between the concentration of cations other than Na^+, and the concentration of anions other than Cl^- and $HCO3^-$ in the plasma and it usually increases as the accumulation of organic anions such as lactate in the blood. Glucose and lactate concentrations were also altered, with an apparent increase of both compounds in the GHB/L-lactate 121 and 302.5 $mg \bullet hr^{-1}kg^{-1}$ infusions; however, the concentrations were all within the normal ranges (Table 2).

Urine pH changes after GHB and L-lactate treatments

The blank urine pH values averaged 6.6 in the absence of treatment, but increased after GHB administration (control), from 6.88 ± 0.26 at 1 hour to 7.53 ± 0.36 at 3 hours. The urine pH after administering GHB plus L-lactate at the three different doses was also increased significantly from that following GHB treatment alone; the changes in urine pH after L-lactate treatment were dose-dependent. The urine pH values measured after administration of the 3 lactate doses (lowest to highest) were 7.65±0.25, 8.08±0.18 and 7.73±0.19 at 1 hour and 8.19±0.13, 7.81±0.63 and 8.56±0.18 at 3 hours.

Effects of combined D-mannitol-L-lactate treatment on GHB toxicokinetics

The steady-state plasma concentrations of GHB were significantly decreased after the administration of L-lactate 60.5 $mg \bullet hr^{-1}kg^{-1}$ and the co-administration of D-mannitol and L-lactate (60.5 $mg \bullet hr^{-1}kg^{-1}$); however the steady-state plasma concentration of GHB following the

administration of D-mannitol was not different from that of the control (Table 1). The total and renal clearances of GHB in the combined D-mannitol and L-lactate treatment group were similar to those of the L-lactate group, but were significantly higher than those of the control group (Fig 2). The metabolic clearances and GFR values were not significantly different in all groups (Fig 2). Consistent with the changes in renal clearance, the amount of GHB reabsorbed was significantly decreased after L-lactate treatment and combined D-mannitol and L-lactate treatment, but not after D-mannitol treatment (Table 1). The total brain concentrations of GHB after the 3-hour infusion were determined (Table 1). There were no significant differences among the brain concentrations observed in the control and treatment groups.

pH and electrolyte concentrations in blood after combined L-lactate/ D-mannitol treatment

The blood pH for the GHB treatment group was not changed significantly; however, the blood pH of the combined L-lactate/D-mannitol group was increased significantly from pH 7.44 to 7.49 (Table 2). The concentrations of Na^+, K^+, and Ca^{2+} were not significantly changed with a 3-hour infusion of GHB alone (control group). The Cl^- concentration was significantly decreased after a 3-hour GHB infusion, but the concentration was still within the normal range. D-mannitol, L-lactate and combined L-lactate/D-mannitol treatments had similar effects on the concentrations of Na^+, Cl^-, K^+, and Ca^{2+} as observed in the control group (Table 2). Anion gaps and glucose concentrations were slightly increased with the infusion of GHB alone and the combination groups, but the concentrations were still within the normal range (Table 2).

Urine pH and volumes changes after combined L-lactate/D-mannitol treatment

The urine pH values in the D-mannitol treatment group at 1 and 3 hours were 6.92 ± 0.41 and 7.48 ± 0.32, respectively (Fig 3). The urine pH values at 1 and 3 hours after the treatment with L-lactate/D-mannitol at 1 and 3 hours were 7.34 ± 0.53 and 8.02 ± 0.24, respectively, which were higher than that of the control (Fig 3A). The urine volumes obtained in the L-lactate alone or D-mannitol-lactate combined groups were significantly higher than that of the control group (Fig 3B), likely due to the large volumes of fluid administrated during the experiments (the total volumes of infusion solution were 9.6, 9.6, 12.6, and 12.6 ml for control, D-mannitol only, L-lactate only, and D-mannitol-L-lactate groups, respectively). There was no significant change in urine volume after D-mannitol administration compared to that in the control group (Fig 3B).

Effects of Lactated Ringer's solution and D-mannitol administration on GHB TK and TD

To further develop a clinically relevant detoxification treatment, Lactated Ringer's solution, which consists of 28 mM lactate, was given to rats by *iv* infusion (300 μl/min) either alone or together with D-mannitol (0.5 g/kg). GHB (1000 mg/kg) was given as an iv bolus to all groups in order to study the effect of interventions on the toxicodynamics (hypotic effect) of GHB. As shown in Fig.4, compared to the control group (GHB infused with saline), administration of Lactated Ringer's solution, D-mannitol, or Lactated Ringer's solution/D-mannitol all resulted in a decrease in GHB plasma concentrations. However, a significant decrease in the GHB AUC was only observed in the combined treatment group (109 ± 24.1 in control vs. 69.6 ± 1.00 $mg \bullet ml^{-1} \bullet min$, $p < 0.05$). The total and renal clearances of GHB in the D-mannitol and combined treatment groups, but not the Lactated Ringer's solution group, were significantly higher than

those of the control group (Table 3). Compared with the control group, the metabolic clearance value in the combined treatment group was significantly increased as well (355 ± 56.6 in control vs. 484 ± 19.6 mg• hr^{-1} kg^{-1}, $p < 0.05$) (Table 3).

In the control group, the time of loss of the righting reflex (LRR, an indicator of the onset of sleep), the time of regaining of the righting reflex (RRR, an indicator of the offset of sleep), and total sleep time of rats were 3 ± 1, 131 ± 40, and 128 ± 41 minutes, respectively. Compared with the control group, administration of Lactated Ringer's solution, or D-mannitol, or Lactated Ringer's solution/D-mannitol delayed the onset of GHB-induced sleep and decreased total sleep time. However, statistically significant changes were detected only for the combined treatment group (Table 3). At the return of righting reflex time, there was no significant difference in GHB plasma concentrations among all control and treatment groups (~ 0.32 mg/ml, Table 3), indicating this concentration might represent the wake-up threshold concentration in rats. This further suggested that the hypnotic effect of GHB was closely related to its plasma concentration.

DMD Fast Forward. Published on August 21, 2008 as DOI: 10.1124/dmd.108.022996
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

GHB is an endogenous fatty acid, which is fully ionized at physiological pH. The transport of GHB across various biological barriers and cellular membranes, such as the blood-brain barrier, brain cells and proximal tubule cells requires specific transporters (Benavides et al., 1982; Bhattacharya and Boje, 2004; Wang et al., 2006). The renal transport of GHB has been studied previously, and the monocarboxylate transporter-1 (MCT1) represents an important transporter for GHB transport across proximal tubule cells (Wang et al., 2006). Most of GHB filtered into kidney tubules is reabsorbed back into blood circulation in a capacity-limited manner (Morris et al., 2005); hence, the renal clearance of GHB may be increased by inhibiting its reabsorption using specific transporter inhibitors such as L-lactate and pyruvate (Morris et al., 2005). Overdoses of GHB result in coma, seizure and death: there are no specific antidotes for the treatment of GHB overdoses. Our approach is to devise a mechanistic strategy for the treatment of GHB overdoses using specific inhibitors for MCTs, membrane transporters that are involved in the renal reabsorption of GHB. Therefore, in this study we evaluated the dose-dependent effects of L-lactate or Lactated Ringer's solution, specific inhibitors of MCT-mediated transport, on the TK and TD of GHB. The effects of administering the osmotic diuretic D-mannitol concomitantly with a low dose of L-lactate were also evaluated.

In the present investigation we determined the concentration-dependent effects of L-lactate in order to evaluate the in vivo relationship between plasma lactate concentration and MCT inhibition. A 10-fold lower dose of L-lactate, than used previously (Morris et al., 2005), was found to be effective in increasing the renal (70% increase: 163 ± 48 vs. 96 ± 26 $ml \bullet hr^{-1}kg^{-1}$ in control group, $P<0.01$) and total (30% increase: 441 ± 44 vs. 340 ± 39 $ml \bullet hr^{-1}kg^{-1}$ in control

group, P<0.01) clearances of GHB in this study. When GHB was given by *iv* infusion, the increase of GHB total clearance following the administration of L-lactate was mainly due to an increase in the renal clearance of GHB, and there was no change in the metabolic clearance of GHB. We had previously reported an increase in the metabolic clearance of GHB after L-lactate treatment (Morris et al., 2005), but this was following the administration of a lower dose of GHB by *iv* infusion than used in the present study. With this lower dose of GHB, the capacity-limited metabolic clearance was changed from ~250 to ~500 $ml \bullet hr^{-1}kg^{-1}$ as the plasma concentration of GHB decreased from 0.40 to 0.25 mg/ml. In contrast, in the present study, the plasma concentration of GHB decreased from 0.60 to 0.40 mg/ml, and the metabolic clearance changed from ~240 to ~290 $ml \bullet hr^{-1}kg^{-1}$, indicating that metabolism remained saturated over this concentration range. However, when GHB was given as an *iv* bolus in the current study, a small but significant increase was observed in the metabolic clearance in the L-lactate/D-mannitol treatment group (355 ± 56.6 in control vs. 484 ± 19.6 mg/hr/kg, $p < 0.05$). This is likely due to the greater decrease in the plasma concentration of GHB that occurred with this combined treatment.

The administration of L-lactate or combined L-lactate/D-mannitol produced increases in both blood and urine pH. There were small changes in blood electrolytes, glucose and lactate concentrations, but most values remained within the normal range of values. Miller et al. (Miller et al., 2002) reported that the exogenous administration of L-lactate does not alter the endogenous production and metabolic clearance of L-lactate. The clearance of L-lactate in humans at rest has been reported to be 22.7 to 27 $ml \bullet min^{-1}kg^{-1}$ (Mazzeo et al., 1986; Stanley et al., 1988), which is much lower than that in the rat at rest (180.6 $ml \bullet min^{-1}kg^{-1}$) (Donovan and

Brooks, 1983). A blood L-lactate concentration of 4 mM have been reported following the exogenous administration of 2.76 - 2.98 $mg \bullet min^{-1} kg^{-1}$ in humans (Miller et al., 2002), and this dose is close to the intermediate L-lactate dose used in this study.

Although a significant increase of urine pH after L-lactate treatment was observed, it is unlikely that this change contributed significantly to the inhibition of GHB reabsorption, since the urine pH after GHB in the presence or absence of L-lactate was greater than or equal to 7.2; at this pH value GHB is fully ionized (>99%) so very limited passive reabsorption would be expected. The urine volumes of the L-lactate treatment groups were also significantly higher than that of the controls; however, there were no differences in urine volume among the three L-lactate dose groups although there were concentration-dependent decreases in GHB renal clearance. Overall, the results suggest that the increase in GHB clearance by L-lactate was due to, at least in part, the inhibition of renal transport by MCTs.

In this investigation, the infusion of 3 doses of L-lactate resulted in changes in the plasma concentrations but no change in brain concentrations of GHB. These findings suggest that total brain GHB concentrations are not correlated with GHB concentrations at the site of action. Brain and CSF concentrations have not been extensively examined but Snead (Snead et al., 1980) reported a good association between plasma or CSF concentrations and electroencephalographic and behavioral effects after GHB administration in cats. In previous studies, we have reported that plasma concentrations correlate with the RRR after GHB administration in rats (Wang et al., 2008). Additionally, Kapadia et al. (Kapadia et al., 2007) reported that GHB brain extracellular

fluid concentrations may correlate with GHB hypnotic effects. As well, it is possible that a different neurotransmitter, such as GABA that is formed from GHB in the brain (Hechler et al., 1997), may better correlate with RRR after GHB administration, and therefore should be monitored when investigating concentration-effect relationships.

D-mannitol is an osmotic diuretic agent and the dose of D-mannitol (0.5 g/kg) used in this study should provide plasma concentrations of D-mannitol necessary for its diuretic effect. Clinically, D-mannitol is administered via *iv* bolus or infusion with doses ranging from 0.25 – 1.0 g/kg body weight, administered as 15% or 20% solutions (Knapp, 2005). In this investigation, the urine volumes of the D-mannitol treatment groups were not significantly different from those of the control groups. This finding may be a result of our study protocol, where a relatively large volume of infusion solution was administered resulting in diuresis. The renal and total clearances of GHB were not significantly increased by D-mannitol. We have previously reported that higher doses of D-mannitol can result in increases in the renal and total clearances of GHB (Morris et al., 2005); therefore, osmotic diuresis may be effective in increasing the renal clearance of GHB at higher doses of D-mannitol. Interestingly, when GHB was given as an *iv* bolus, a small but significant increase in the renal and total clearances of GHB was observed in the D-mannitol treatment group, but not the Lactated Ringer's solution treatment group (Table 3). This is probably due to the different dosing regimen of GHB in these studies (*iv* infusion to steady state vs. *iv* bolus injection).

An interesting phenomenon was observed when we combined D-mannitol (0.5 g/kg) with Lactated Ringer's solution: the AUC of GHB and sleep time were significantly decreased when

compared with the control group. However, these effects were not statistically significant when only Lactated Ringer's Solution or D-mannitol was administered. One possible explanation is that L-lactate and D-mannitol acted additively or synergistically to increase GHB renal clearance. As a result, GHB plasma concentrations are significantly decreased, which may reflect changes in GHB at the site of action in the brain. Additionally, other mechanisms may also contribute to the additive/synergistic effects of mannitol and L-lactate on GHB TD, including changes in GHB uptake into the brain and changes in L-lactate disposition. The mechanism underlying the enhanced effects on the TK and TD of GHB is unknown.

In summary, we have characterized the effects of administering a MCT inhibitor, L-lactate (or Lactated Ringer's solution), on GHB toxicokinetics and toxicodynamics. The administration of L-lactate (or Lactated Ringer's solution), alone and with concomitant D-mannitol, resulted in a significant effect on GHB toxicokinetics and toxicodynamics. The strategies used in this study may represent potential clinical approaches for the treatment of GHB overdoses and a general strategy to treat overdoses of other small molecular weight organic anions.

Acknowledgements

We are grateful to Mr. David Soda and Ms. Sunmi Fung for their technical support.

References

Andriamampandry C, Taleb O, Viry S, Muller C, Humbert JP, Gobaille S, Aunis D and Maitre M (2003) Cloning and characterization of a rat brain receptor that binds the endogenous neuromodulator gamma-hydroxybutyrate (GHB). *Faseb J* **17**:1691-1693.

Arena C and Fung HL (1980) Absorption of sodium gamma-hydroxybutyrate and its prodrug gamma-butyrolactone: relationship between in vitro transport and in vivo absorption. *J Pharm Sci* **69**:356-358.

Benavides J, Rumigny JF, Bourguignon JJ, Wermuth CG, Mandel P and Maitre M (1982) A high-affinity, Na+-dependent uptake system for gamma-hydroxybutyrate in membrane vesicles prepared from rat brain. *J Neurochem* **38**:1570-1575.

Bernasconi R, Lauber J, Marescaux C, Vergnes M, Martin P, Rubio V, Leonhardt T, Reymann N and Bittiger H (1992) Experimental absence seizures: potential role of gamma-hydroxybutyric acid and GABAB receptors. *J Neural Transm Suppl* **35**:155-177.

Bhattacharya I and Boje KM (2004) GHB (gamma-hydroxybutyrate) carrier-mediated transport across the blood-brain barrier. *J Pharmacol Exp Ther* **311**:92-98.

Carai MA, Colombo G, Brunetti G, Melis S, Serra S, Vacca G, Mastinu S, Pistuddi AM, Solinas C, Cignarella G, Minardi G and Gessa GL (2001) Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. *Eur J Pharmacol* **428**:315-321.

Darling IM and Morris ME (1991) Evaluation of "true" creatinine clearance in rats reveals extensive renal secretion. *Pharm Res* **8**:1318-1322.

Davidson WD and Sackner MA (1963) Simplification of the Anthrone Method for the Determination of Inulin in Clearance Studies. *J Lab Clin Med* **62**:351-356.

Donovan CM and Brooks GA (1983) Endurance training affects lactate clearance, not lactate production. *Am J Physiol* **244**:E83-92.

Ferrara SD, Zotti S, Tedeschi L, Frison G, Castagna F, Gallimberti L, Gessa GL and Palatini P (1992) Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br J Clin Pharmacol* **34**:231-235.

Ferrier B, Martin M, Janbon B and Baverel G (1992) Transport of beta-hydroxybutyrate and acetoacetate along rat nephrons: a micropuncture study. *Am J Physiol* **262**:F762-769.

Fishbein WN and Bessman SP (1964) Gamma-Hydroxybutyrate in Mammalian Brain. Reversible Oxidation by Lactic Dehydrogenase. *J Biol Chem* **239**:357-361.

Fung HL, Haas E, Raybon J, Xu J and Fung SM (2004) Liquid chromatographic-mass spectrometric determination of endogenous gamma-hydroxybutyrate concentrations in rat brain regions and plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* **807**:287-291.

Gallimberti L, Spella MR, Soncini CA and Gessa GL (2000) Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. *Alcohol* **20**:257-262.

Halestrap AP and Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem J* **343 Pt 2**:281-299.

Hechler V, Ratomponirina C and Maitre M (1997) gamma-Hydroxybutyrate conversion into GABA induces displacement of GABAB binding that is blocked by valproate and ethosuximide. *J Pharmacol Exp Ther* **281**:753-760.

Kapadia R, Bohlke M and Maher TJ (2007) Detection of gamma-hydroxybutyrate in striatal microdialysates following peripheral 1,4-butanediol administration in rats. *Life Sci* **80**:1046-1050.

Knapp JM (2005) Hyperosmolar therapy in the treatment of severe head injury in children: mannitol and hypertonic saline. *AACN Clin Issues* **16**:199-211.

Lettieri JT and Fung HL (1979) Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. *J Pharmacol Exp Ther* **208**:7-11.

Maitre M (1997) The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol* **51**:337-361.

Mamelak M, Scharf MB and Woods M (1986) Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. *Sleep* **9**:285-289.

Mason PE and Kerns WP, 2nd (2002) Gamma hydroxybutyric acid (GHB) intoxication. *Acad Emerg Med* **9**:730-739.

Mazzeo RS, Brooks GA, Schoeller DA and Budinger TF (1986) Disposal of blood [1-13C]lactate in humans during rest and exercise. *J Appl Physiol* **60**:232-241.

Miller BF, Fattor JA, Jacobs KA, Horning MA, Navazio F, Lindinger MI and Brooks GA (2002) Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion. *J Physiol* **544**:963-975.

Morris ME, Hu K and Wang Q (2005) Renal clearance of gamma-hydroxybutyric acid in rats: increasing renal elimination as a detoxification strategy. *J Pharmacol Exp Ther* **313**:1194-1202.

Nelson T, Kaufman E, Kline J and Sokoloff L (1981) The extraneural distribution of gamma-hydroxybutyrate. *J Neurochem* **37**:1345-1348.

Okun MS, Boothby LA, Bartfield RB and Doering PL (2001) GHB: an important pharmacologic and clinical update. *J Pharm Pharm Sci* **4**:167-175.

Schwartz RH, Milteer R and LeBeau MA (2000) Drug-facilitated sexual assault ('date rape'). *South Med J* **93**:558-561.

Shannon M and Quang LS (2000) Gamma-hydroxybutyrate, gamma-butyrolactone, and 1,4-butanediol: a case report and review of the literature. *Pediatr Emerg Care* **16**:435-440.

Snead OC, 3rd, Bearden LJ and Pegram V (1980) Effect of acute and chronic anticonvulsant administration on endogenous gamma-hydroxybutyrate in rat brain. *Neuropharmacology* **19**:47-52.

Stanley WC, Wisneski JA, Gertz EW, Neese RA and Brooks GA (1988) Glucose and lactate interrelations during moderate-intensity exercise in humans. *Metabolism* **37**:850-858.

Tedeschi L, Carai MA, Frison G, Favretto D, Colombo G, Ferrara SD and Gessa GL (2003) Endogenous gamma-hydroxybutyric acid is in the rat, mouse and human gastrointestinal tract. *Life Sci* **72**:2481-2488.

Wang Q, Darling IM and Morris ME (2006) Transport of gamma-hydroxybutyrate in rat kidney membrane vesicles: Role of monocarboxylate transporters. *J Pharmacol Exp Ther* **318**:751-761.

Wang X, Wang Q and Morris ME (2008) Pharmacokinetic interaction between the flavonoid luteolin and gamma-hydroxybutyrate in rats: potential involvement of monocarboxylate transporters. *Aaps J* **10**:47-55.

Footnotes:

a) This work was supported in part by NIH Grant DA023223 .

b) Corresponding author is MEM**.**

[1]Current address for Qi Wang is Bristol Myers Squibb, Mailstop HW17 1.07, 311 Pennington Rocky-Hill Rd, Pennington, NJ 08534. Current address for Xiaodong Wang is Bristol Myers Squibb, Mailstop HW8 1.18, 311 Pennington Rocky-Hill Rd, Pennington, NJ 08534.

Figure Legends:

Figure 1. The renal clearance (CL_R), metabolic clearance (CL_m), and total clearance (CL_T) of GHB determined after the infusion of different doses of L-lactate. The control was administered GHB (208 mg/kg/hr) alone. L-lactate-1, L-lactate-2, and L-lactate-3 represent the doses of 60.5, 121, and 302.5 $mg \bullet hr^{-1} \bullet kg^{-1}$, respectively. Results are plotted as mean ± SD, n = 3-5. Statistical differences were compared with the control ** $P<0.01$, *** $P<0.001$, using a one-way ANOVA followed by a Dunnett's post hoc test.

Figure 2. The renal clearance (CL_R), metabolic clearance (CL_m) and total clearance (CL_T) of GHB determined after the concomitant treatment with D-mannitol, L-lactate or D-mannitol/L-lactate. The control was administered with GHB (208 $mg \bullet hr^{-1}kg^{-1}$) only. L-lactate was administered at a dose of 60.5 $mg \bullet hr^{-1}kg^{-1}$ by *iv* infusion; D-mannitol was administered as an *iv* bolus dose of 0.5g/kg; Man+Lact represents the combined treatment with D-mannitol and L-lactate. One-way ANOVA followed by Dunnett's test, n =3-8, mean ± SD. **$P<0.01$.

Figure 3. Effect of treatment with GHB alone or GHB with L-lactate, GHB with D-mannitol, or GHB with the combination of D-mannitol/L-lactate on urine pH and volume. Urine pH changes (A) and urine volume changes (B) after administration of L-lactate 60.5 $mg \bullet hr^{-1}kg^{-1}$, D-mannitol 0.5 g/kg, and the combination of D-mannitol and L-lactate. One-way ANOVA followed by Dunnett's test, n =3-5, mean ± SD. *$P<0.05$, **$P<0.01$.

Figure 4. Plasma concentrations of GHB after treatment with GHB alone or with concomitant Lactated Ringer's solution, D-mannitol, or Lactated Ringer's solution/D-mannitol administration.

The control was administered with GHB (1000 mg/kg, *iv* bolus) only, followed by *iv* infusion of saline at the rate of 300 μl/min; L-lactate represents the *iv* infusion of Lactated Ringer's solution at the rate of 300 μl/min; D-mannitol represents the 0.5g/kg bolus dose of D-mannitol; Man+Lact represents the combined treatment with D-mannitol 0.5g/kg and iv infusion of Lactated Ringer's solution at the rate of 300 μl/min, n = 3-4 per group.

Table 1. Effects of L-lactate, mannitol and combined mannitol/L-lactate administrations on GHB toxicokinetics.

	Control	L-lactate (60.5 $mg\bullet hr^{-1}kg^{-1}$)	L-lactate (121 $mg\bullet hr^{-1}kg^{-1}$)	L-lactate (302.5 $mg\bullet hr^{-1}kg^{-1}$)	D-mannitol (0.5 g/kg)	D-mannitol and L-lactate
Plasma [GHB] (mg/ml)	0.62 ± 0.11	0.48 ± 0.04**	0.46 ± 0.01**	0.38 ± 0.02**	0.57 ± 0.08	0.45 ± 0.06**
GHB f_e (%)	32.5 ± 5.2	36.6 ± 9.5	48.7 ± 2.7	41.8 ± 4.4	31.9 ± 6.5	39.8 ± 14.5
Plasma [Inulin] (mg/ml)	0.19 ± 0.02	0.23 ± 0.04	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.02	0.19 ± 0.04
GFR ($ml\bullet hr^{-1}\ kg^{-1}$)	584 ± 111	482 ± 54	603 ± 30	549 ± 16	623 ± 114	538 ± 87
% of GHB reabsorbed	80.3 ± 2.6	66.3 ± 10.2**	63.59 ± 5.1**	61.1 ± 4.8**	83.1 ± 6.5	65.1 ± 13.3*
Brain [GHB] (mg/g)	0.08±0.04	0.10±0.02	0.09±0.01	0.07±0.02	0.10±0.04	0.06±0.02
[L-lactate] change (mM)	0 ± 0.08	0.27 ± 0.15	0.43 ± 0.06**	0.70 ± 0.08**	0.00 ± 0.01	0.28 ± 0.23

GHB (208 $mg\bullet hr^{-1}\ kg^{-1}$) was administered by iv infusion to reach steady-state concentration. L-lactate (60.5, 121, and 302 $mg\bullet hr^{-1}kg^{-1}$) was administered by *iv* infusion for 3 hours. D-mannitol (0.5g/kg) was administered by *iv* bolus. For the combined treatment, L-lactate (60.5 $mg\bullet hr^{-1}kg^{-1}$) was administered by *iv* infusion for 3 hours and D-mannitol (0.5g/kg) was administered by *iv* bolus. The ratio of brain to plasma was calculated using the brain concentrations divided by the last plasma concentration of GHB at the end of the study. [L-lactate] change represents the concentration difference between the beginning and the end of the treatments; the mean L-

lactate concentrations are given in Table 2. One-way ANOVA followed by Dunnett's test, n = 3-8, mean ± SD. * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

Table 2. Blood pH and electrolyte concentrations before and after GHB administration alone or with concomitant L-lactate, and/or D-mannitol administrations.

	GHB only		L-lactate (60.5 mg•hr^{-1}kg^{-1})		L-lactate (121 mg• hr^{-1}kg^{-1})		L-lactate (302.5 mg• hr^{-1}kg^{-1})		D-Mannitol (0.5 g/kg)		D-Mannitol + L-Lactate	
	0 min	180 min	0 min	180 min	0 min	180 min	0 min	180 min	0min	180min	0min	180min
pH	7.44±0.02	7.48±0.02	7.43± 0.01	7.52± 0.01**	7.46±0.01	7.52 ± 0.02*	7.46± 0.01	7.53 ±0.01***	7.46± 0.01	7.48± 0.03*	7.44± 0.01	7.49± 0.01***
K^+ (mM)	4.1± 0.5	3.7± 0.4	4.2± 0.4	4.0± 0.4	4.5± 0.7	4.0± 0.7	3.8± 0.2	3.5± 0.2	4.0± 0.2	3.4±0.2*	4.2±0.5	3.6±0.3*
Na^+ (mM)	145± 2	147± 2	145± 2	145± 2	145± 2	145± 2	145± 2	146± 3	141±1	143±1	142±1	143±2
Cl^- (mM)	108± 2	99± 2**	107± 2	97± 2**	107± 3	97± 2*	108± 4	95± 1**	104±2	100±1	106±4	96±3***
Ca^{2+} (mM)	0.98± 0.20	0.96± 0.12	0.90± 0.17	0.77± 0.20	0.79± 0.14	0.80± 0.04	0.82± 0.09	0.96± 0.31	1.22± 0.06	1.17± 0.04	1.25± 0.09	1.16± 0.17
Anion-gap (mM)	14.9± 2.4	19.8±3.0*	17.5± 2.2	19.5± 2.0	16.7± 2.1	18.3± 1.8	14.2± 2.7	17.1± 3.7*	13.3± 0.8	16.7± 0.3	12.7± 0.9	16.2±2.4*
Glucose (mM)	7.5±0.6	9.5±1.6*	8.1±0.3	7.3±1.2	7.0±0.2	9.3±0.8*	6.5±0.7	8.9±0.7**	8.4± 1.8	10.0±1.2	6.7± 0.8	9.5± 2.0*
L-lactate (mM)	1.1±0.2	1.1±0.2	1.1±0.2	1.4±0.5	1.0±0.1	1.4± 0.1**	0.8±0.1	1.4± 0.2**	1.2± 0.4	0.9± 0.1	1.0± 0.3	1.3± 0.4

GHB (208 mg•hr^{-1} kg^{-1}) was administered by *iv* infusion to reach steady-state concentration. L-lactate (60.5, 121, and 302 mg•hr^{-1}kg^{-1}) was administered by iv infusion for 3 hours. D-mannitol (0.5g/kg) was administered by *iv* bolus. For the combined treatment, L-lactate (60.5 mg•hr^{-1}kg^{-1}) was administered by *iv* infusion for 3 hours and D-mannitol (0.5g/kg) was administered by *iv* bolus. Blood pH and electrolyte concentrations before and after the infusion were measured using an ABL 605 Radiometer. The results are

expressed as mean ± SD, n = 3-5. A paired t-test was used to detect statistical significance. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Effects of combined D-mannitol (0.5 g/kg, *iv* bolus) and Lactated Ringer's solution (300 µl/min, *iv* infusion) on GHB toxicokinetics and toxicodynamics.

	Control	D-mannitol (0.5 g/kg)	Lactated Ringer's Solution (300 µl/min)	D-mannitol and Ringer's Solution
AUC [GHB] (mg/ml•min)	109 ± 24.1	77.5 ± 9.24	83.5 ± 13.5	69.6 ± 1.00*
Total clearance (ml/hr/kg)	569 ± 113	781 ± 87.3*	733 ± 120	862 ± 12.3**
Renal clearance (ml/hr/kg)	215 ± 64.9	356 ± 25.6*	329 ± 85.0	378 ± 8.61*
Metabolic clearance (ml/hr/kg)	355 ± 56.6	425 ± 64.8	404 ± 41.0	484 ± 19.6*
GHB f_e (%)	37.2 ± 5.17	45.8 ± 2.62	44.4 ± 4.83	43.8 ± 1.53
LRR (min)	3 ± 1	4 ± 1	7 ± 3	13 ± 2**
RRR (min)	131 ± 40	93 ± 3	93 ± 17	75 ± 3*
Sleep time (min)	128 ± 41	89 ± 4	86 ± 20	62 ± 2*
C_{RRR} (mg/ml)	0.322 ± 0.052	0.315 ± 0.040	0.315 ± 0.014	0.352 ± 0.005

GHB (1000 mg/kg) was administered by *iv* bolus. Saline or Lactated Ringer's solution was administered by *iv* infusion at the rate of 300 µl/min for 4 hours. D-mannitol (0.5g/kg) was administered by *iv* bolus. For the combined treatment group, Lactated Ringer's solution was administered by *iv* infusion (300 µl/min) for 4 hours and D-mannitol (0.5g/kg) was administered by *iv* bolus. One-way

ANOVA followed by Dunnett's test was used to detect statistical significance, n = 3-4, mean ± SD. * $p<0.05$, ** $p<0.01$. LRR: loss of the righting reflex, RRR: regaining of righting reflex. C_{RRR}: Plasma concentration of GHB at the time point of RRR.

Figure 1

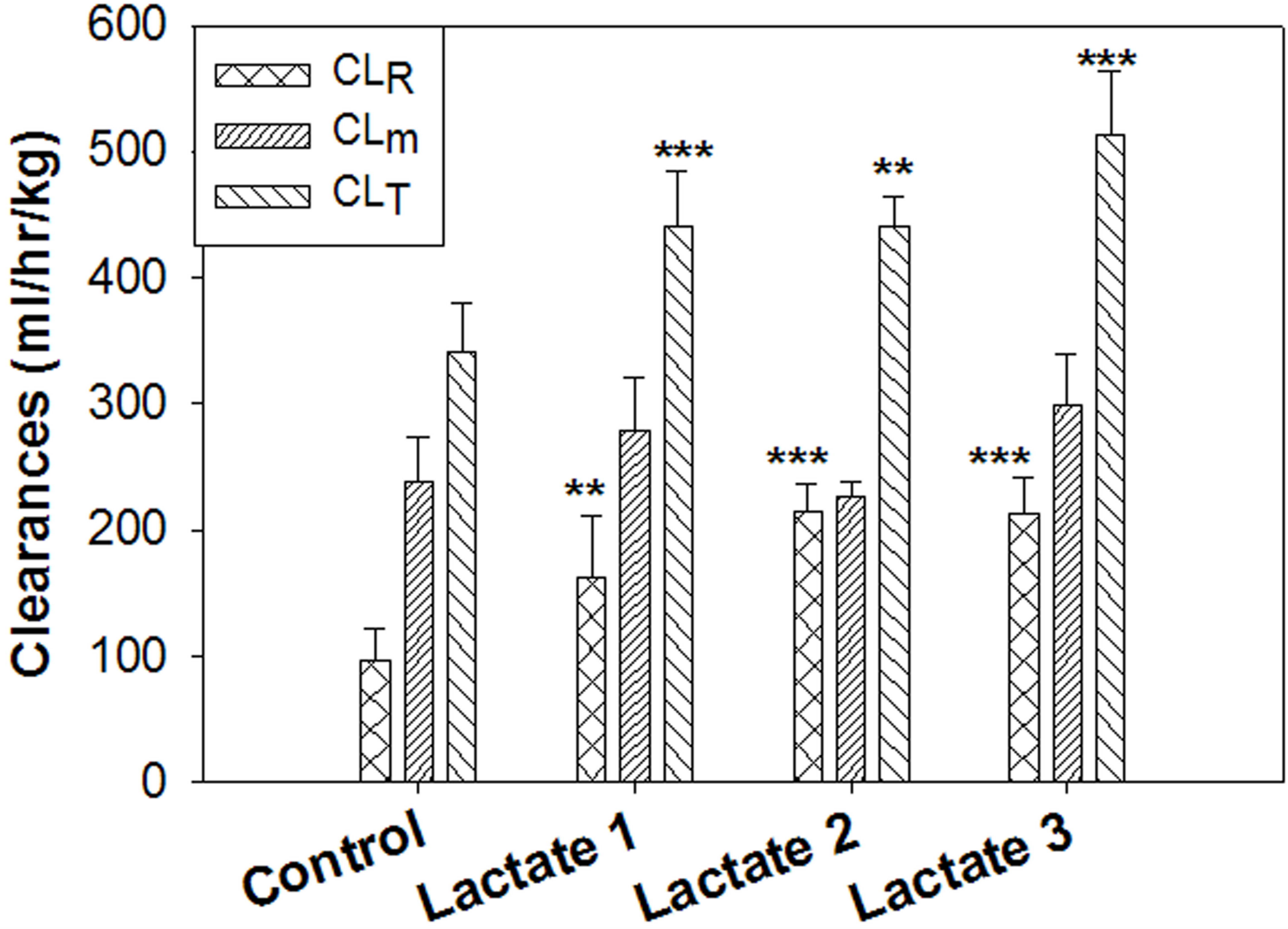

600
500
400
300
200
100
0
Clearances (ml/hr/kg)
CLR
CLm
CLT
**
***
***
**
***
***
Control
Lactate 1
Lactate 2
Lactate 3

Figure 2

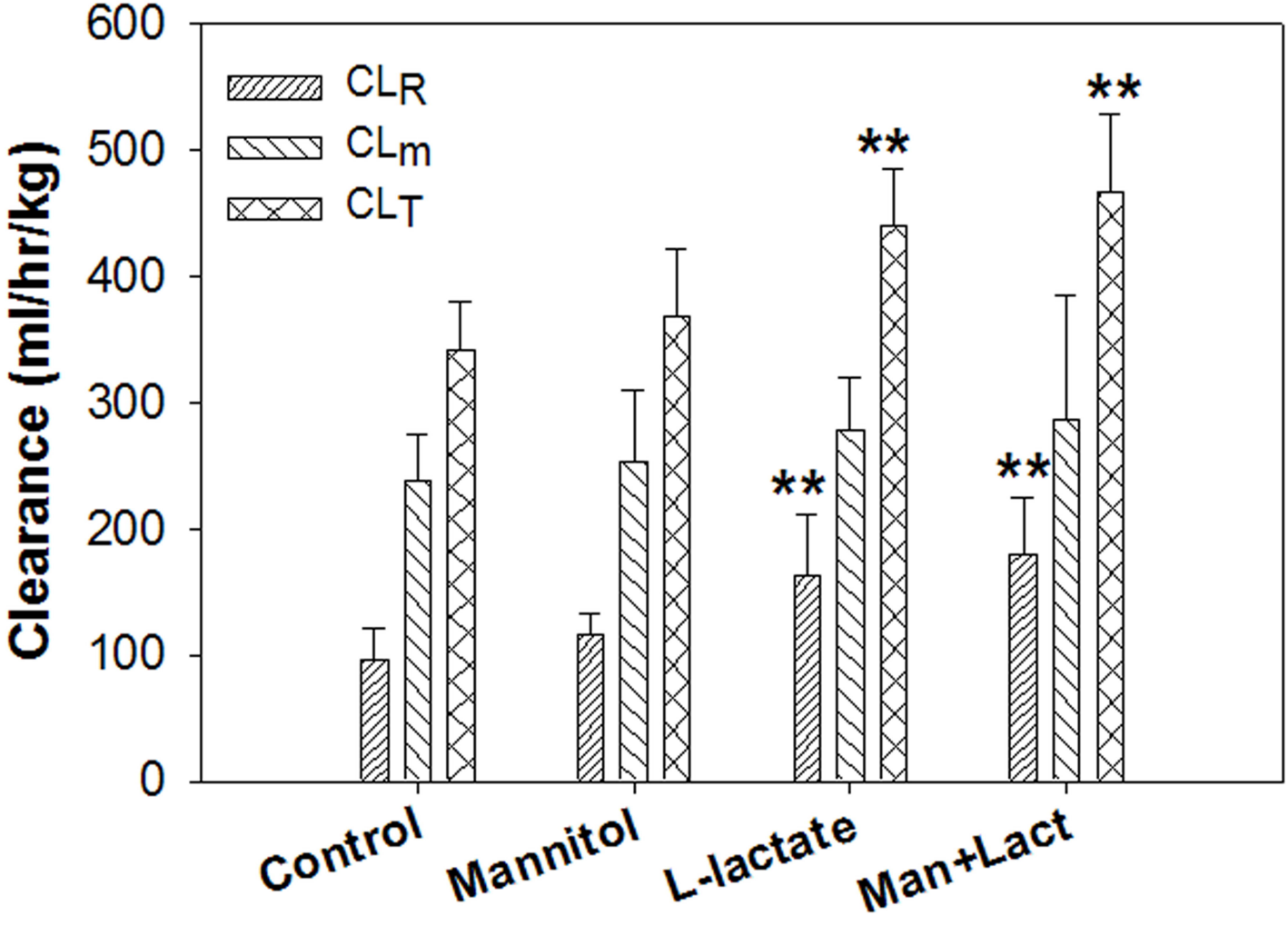

600
500
400
300
200
100
0
Clearance (ml/hr/kg)
CLR
CLm
CLT
**
**
**
Control
Mannitol
L-lactate
Man+Lact

Figure 3

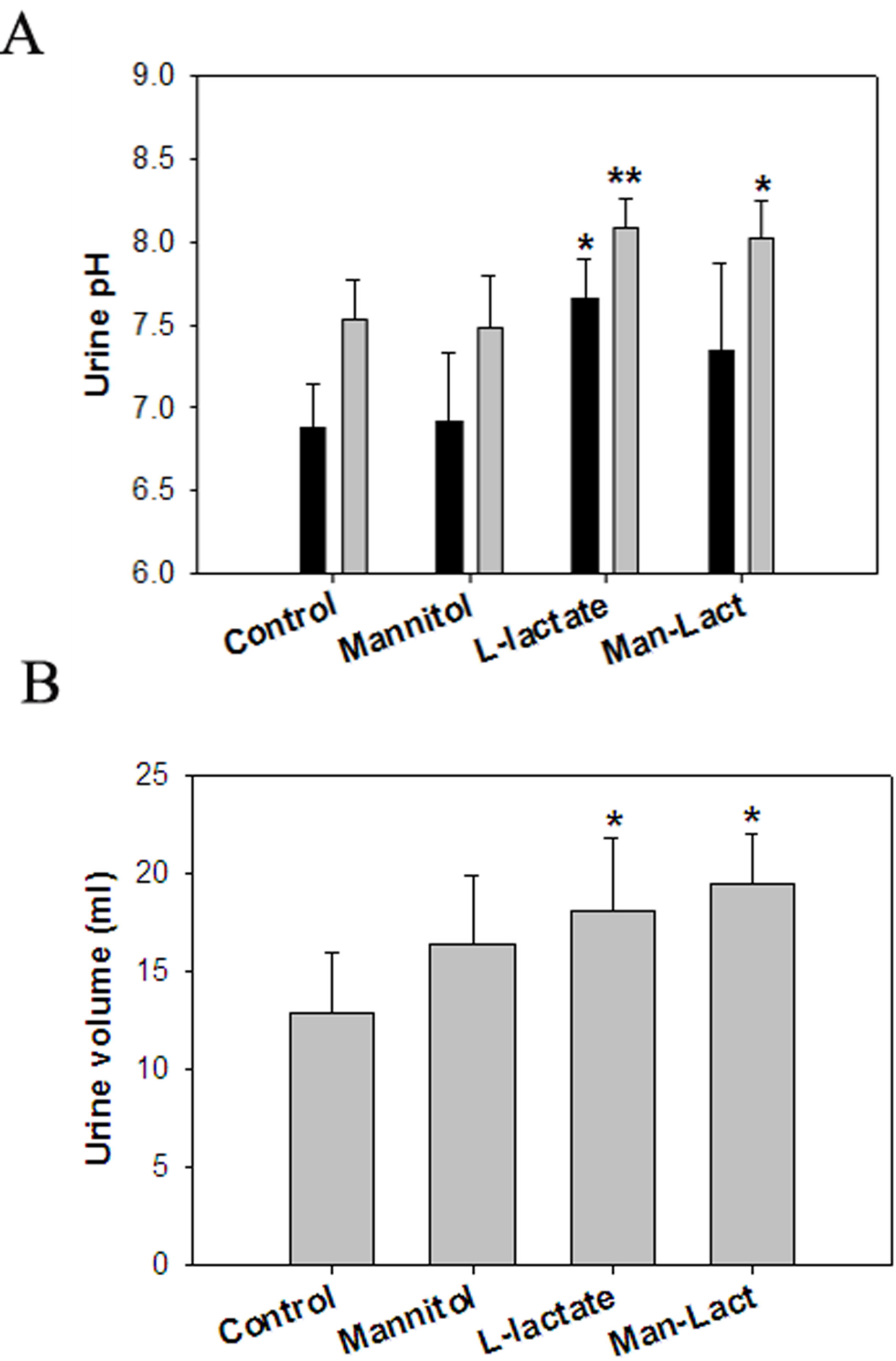
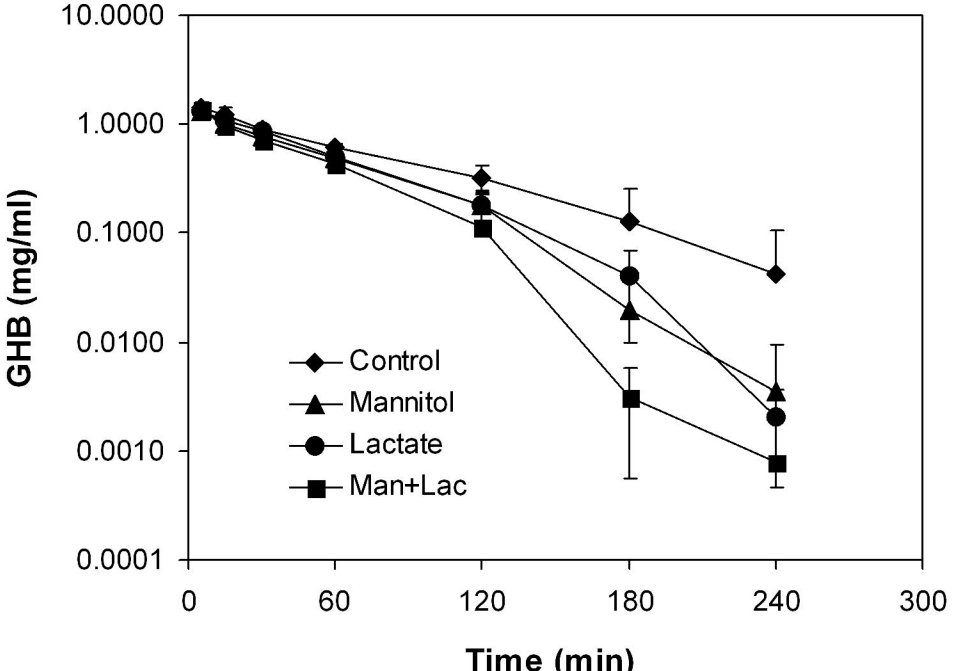

A
9.0
8.5
8.0
7.5
7.0
6.5
6.0
Urine pH
*
**
*
Control
Mannitol
L-lactate
Man-Lact
B
25
20
15
10
5
0
Urine volume (ml)
*
*
Control
Mannitol
L-lactate
Man-Lact

Fig. 4


10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
GHB (mg/ml)
Control
Mannitol
Lactate
Man+Lac
0
60
120
180
240
300
Time (min)