Published in final edited form as:

J Pharm Sci. 2014 October ; 103(10): 3310–3315. doi:10.1002/jps.24122.

Effect of 3, 4-methylenedioxymethamphetamine (MDMA) on the toxicokinetics and sedative effects of the drug of abuse, γ -hydroxybutyric acid

Nisha Vijay and Marilyn E. Morris

Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, SUNY, Buffalo, NY 14214-8033

Abstract

γ -hydroxybutyric acid (GHB) is widely abused in combination with other club drugs such as 3,4-methylenedioxy methamphetamine (MDMA). The objectives of this study were to characterize the effects of MDMA on GHB toxicokinetics/toxicodynamics (TK/TD) and evaluate the use of monocarboxylate transporter (MCT) inhibition as a potential treatment strategy for GHB overdose when GHB is abused with MDMA. Rats were administered GHB 400 mg/kg i.v. alone or with MDMA (5 mg/kg i.v). Effects of MDMA and of the monocarboxylate transporter (MCT) inhibitor, L-lactate, on GHB TK and sedative effects were evaluated. The results of this study demonstrated no significant effect of MDMA on GHB TK or TD. GHB plasma concentrations were unchanged, and GHB concentration-effect relationships, based on plasma and brain concentrations and the return to righting reflex (RRR), were similar in the presence and absence of MDMA. L-lactate administration resulted in a significant decrease in the sedative effect (RRR) of GHB when it was co-administered with MDMA. Our results indicate that MDMA does not affect the TK/TD of GHB at the doses used in this study, and MCT inhibition using L-lactate, an effective overdose treatment strategy for GHB alone, is also effective for GHB overdose when GHB is co-ingested with MDMA.

Keywords

monocarboxylate transporter inhibitor; γ -hydroxybutyrate; GHB; MDMA; overdose treatment; L-lactate

Introduction

γ -hydroxybutyric acid is widely abused as a recreational drug at nightclubs and raves for its euphoric effects. Its overdose can lead to serious adverse effects including hypothermia, respiratory depression, coma and death^{1–3}. In a recreational setting, GHB is typically not ingested alone and co-ingestion with ethanol and other club drugs occurs in the majority of cases^{3–5}. A recent survey of 131 GHB users found that 3,4-methylenedioxymethamphetamine (MDMA) was co-ingested by 51% of the users and an

increased risk of hospital treatment was reported following MDMA co-ingestion⁴. MDMA is a member of the class of amphetamines and is a widely abused psychostimulant drug as it elicits a feeling of euphoria and well-being. It is typically ingested orally and is very often co-abused with GHB^{6,7}. It exhibits nonlinear pharmacokinetics both in humans and rodents^{8–10}. It is postulated that MDMA can lead to autoinhibition of CYP2D6, the enzyme responsible for its own metabolism which is the cause of nonlinearity in its pharmacokinetics¹¹. Overdose of MDMA can lead to severe hyperthermia, rhabdomyolysis, hepatotoxicity and renal failure that can also result in death¹². Hepatotoxicity has been reported as one of the major clinical effects of intoxication of MDMA. Upreti *et al* reported toxic interactions between ethanol and MDMA where a 35% decrease in the enzyme activity of aldehyde dehydrogenase was observed that resulted in accumulation of acetaldehyde and hepatocellular damage¹³. In addition, MDMA has also been shown to cause mechanism-based inhibition of CYP2D6^{14,15}. GHB users have been reported to frequently combine GHB with MDMA with the intention to extend the high associated with MDMA and produce a more intense effect than each drug alone¹⁶. A retrospective case study in 65 GHB intoxications showed a significantly longer time to regain consciousness in individuals who had co-ingested MDMA along with GHB¹⁷. The metabolism of GHB is complex and both mitochondrial and cytosolic enzymes are involved. The rate-limiting step is reported to be the formation of succinic semialdehyde (SSA), a process involving GHB dehydrogenase in the cytosol and GHB transhydrogenase in the mitochondria¹⁸. However, the effect of MDMA on GHB toxicokinetics currently remains unknown.

GHB exhibits nonlinear toxicokinetics due to capacity-limited absorption, metabolism and renal clearance^{19–21}. GHB is a substrate for monocarboxylate transporters (MCTs) that mediate its transport in the kidney, intestine, and brain^{22–25}. The nonlinearity in renal clearance has been attributed to the saturation of active renal reabsorption of GHB in the kidney mediated by MCTs. A recent study showed that MDMA could result in a slight increase in butyrate uptake (a MCT1 substrate) in Caco-2 cells and was also shown to modulate MCT1 expression when the cells were treated with MDMA chronically²⁶. This suggests that MDMA may alter the toxicokinetics/toxicodynamics (TK/TD) of GHB by altering its MCT-mediated transport. However, our knowledge on the TK/TD interactions between GHB and MDMA is currently limited.

At this time, there is no antidote to treat GHB or MDMA overdose when taken alone or in combination and treatment remains to be supportive care. Our laboratory has evaluated MCT inhibition as a potential treatment strategy for GHB overdose by inhibiting its active renal reabsorption mediated by MCTs^{21,27,28}. We have demonstrated that the administration of MCT inhibitors with GHB increases its renal and total clearance and decreases its sedative/hypnotic effects and improves respiratory depression in rats^{27,28}. However, the efficacy of MCT inhibition for the treatment of GHB overdose in the presence of MDMA needs to be evaluated.

The objective of this research was to characterize the effects of MDMA on GHB TK/TD using the end point of sedation and to evaluate the use of MCT inhibition as a potential treatment strategy for GHB overdose when it is co-administered with MDMA.

Materials and Methods

Chemicals and Reagents—Sodium GHB and 3,4-methylenedioxymethamphetamine hydrochloride used in these studies was provided by the National Institute on Drug Abuse. Sodium L-lactate was purchased from Sigma-Aldrich (St. Louis, MO). Deuterated GHB (GHB-d₆) was purchased from Cerilliant Corporation (Round Rock, TX). High-performance liquid chromatography grade acetonitrile and acetic acid were purchased from Honeywell Burdick & Jackson (Muskegon, MI).

Animals and Surgery—Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 270 to 330 g were used for all experiments. All animal procedures were approved by University at Buffalo Institutional Animal Care and Use Committee. Animals were housed under controlled temperature and humidity with an artificial 12 hour light/dark cycle, and food was available ad libitum. Animals were allowed to acclimate to their environment for a minimum of 1 week before surgery. The jugular and femoral vein cannulae were surgically implanted under anesthesia with ketamine/xylazine (75/10 mg/kg). Cannulae were flushed daily with 40 IU/ml heparinized saline to maintain patency. Rats were allowed a minimum of 72 h for recovery from surgery before conducting experiments.

Toxicokinetic/Toxicokinetic interaction studies

Effect of MDMA on sedative effects of GHB—The effect of MDMA on the sedative effects of GHB was measured using the endpoint of righting reflex, similar to our previous studies²⁹. Rats were administered with GHB 400 mg/kg i.v. in the presence and absence of MDMA (5 mg/kg i.v.) via the jugular vein cannula (n = 3–5 in each treatment group). The duration of sedative/hypnotic effect (sleep time), measured as the difference between the time of loss-of-righting reflex (LRR) and return-to-righting reflex (RRR), was recorded in each group. LRR and RRR are defined as the time at which the animal lost or regained the ability to right itself when placed on its back. Animals were left on their back after LRR and the time at which the animal could right itself was defined as RRR. The animals were euthanized at RRR under isoflurane anesthesia followed by collection of blood and brain samples. Brain samples were immediately frozen in liquid nitrogen and stored at –80°C until analysis. In these studies, GHB was administered as a 200 mg/ml solution in sterile water and MDMA as a 5 mg/ml solution in normal saline.

Effect of MDMA on GHB toxicokinetics—To evaluate the effect of MDMA on GHB toxicokinetics, GHB 400 mg/kg i.v. was administered via the jugular vein cannula alone or in combination with MDMA (5 mg/kg i.v. bolus). Blood samples were collected from the jugular vein cannula at 0, 3, 10, 20, 30, 60, 90, 120, 180, 210, 240, 270, and 300 min after GHB administration. The plasma samples were separated from whole blood by centrifugation at 3,000 g for 15 min at 4°C. All plasma samples were stored at –80°C until analysis. In these studies, GHB was administered as a 300 mg/ml solution in sterile water and MDMA as a 5 mg/ml solution in normal saline.

Effect of treatment strategies

Effect of MCT on sedative effects of GHB in presence of MDMA—To assess MCT inhibition as a potential treatment strategy for improving GHB-induced sedation when it is combined with MDMA, the MCT inhibitor L-lactate (66 mg/kg i.v. bolus followed by 302.5 mg/kg/h i.v. infusion) was administered 5 minutes after GHB-MDMA administration. This dose of L-lactate was chosen to increase plasma L-lactate concentrations by 1–2 mM²⁸. The sleep time was measured as the difference between LRR and RRR in each group. The brain and plasma samples were collected at RRR as described above.

Plasma and Brain Sample Analysis—GHB plasma and urine concentrations were measured using a previously validated liquid chromatography coupled to tandem mass spectrometry assay^{28,29}. Plasma samples were prepared by adding 5 µl of internal standard solution containing GHB-d₆ (125 µg/ml) to 50 µl of sample. Plasma standards and quality controls were prepared by adding 5 µl of internal standard solution containing GHB-d₆ (125 µg/ml) and 5 µl of stock solution containing GHB to 45 µl of blank plasma, and 800 µl of 0.1% formic acid in acetonitrile was added to precipitate the plasma proteins. The samples were vortexed, followed by centrifugation at 10,000g for 20 min at 4°C. An aliquot (750 µl) of the supernatant was aspirated and evaporated under a stream of nitrogen gas. The samples were then reconstituted in 250 µl of aqueous mobile phase. Whole brain samples were homogenized in double-distilled water (4 ml/g of tissue). GHB-d₆ (10 µl of a 200 µg/ml solution) was added to 100 µl of brain homogenate of the sample. GHB standards and quality controls were prepared by the addition of 10 µl of GHB-d₆ (200 µg/ml) and 10 µl of GHB stock solution to 100 µl of blank brain homogenate. Double-distilled water (680 µl) was added to both samples and standards, followed by the addition of 1 ml of acetonitrile in order to precipitate the proteins in the sample. Samples were centrifuged at 10,000g for 20 min and the supernatant was stored at –80°C until analysis.

GHB Cell Uptake Studies

The immortalized rat brain capillary endothelial cell line (RBE4) was kindly provided by Professor P. Couraud (Institut Cochin, Paris). RBE4 cells (passages 39–44) were cultured as described previously³⁰. Briefly, cells were grown at 37°C with 5% CO₂ on Type-I collagen-coated flasks, and media was changed every 2–3 days. The culture medium was 1:1 α-minimum essentials medium/Hams F10 nutrient mixture supplemented with L-glutamine (2.0 mM), geneticin (300 µg/ml), human recombinant fibroblast growth factor (1 ng/ml), gentamicin (50 µg/ml), and 10% V/V fetal bovine serum (FBS). Cells were passaged using 0.25% trypsin/EDTA and seeded on individual 35 × 10 mm collagen-coated wells for uptake studies which were performed 7 days post confluence (the time needed for formation of tight junctions).

To study the effect of MDMA on GHB uptake, cells were washed and equilibrated for 20 min at 37°C with uptake buffer containing 138 mM NaCl, 1.8 mM CaCl₂, 5.4 mM KCl, 0.8 mM MgSO₄, 1.0 mM Na₂HPO₄, 5.5 mM D-glucose, and 20 mM HEPES (pH 7.4). Cells were then equilibrated to room temperature for 5 min followed by pre-incubation with MDMA (0.1, 1, or 10 µM) at 37°C for 2 h before incubation with 3 mM [³H]-GHB for 15 s. Previous work indicated that 15 s is within the time of linear uptake of GHB in RBE4

cells²⁵. After incubation with [³H]-GHB, cells were washed 3 times with ice-cold uptake buffer and lysed with 0.5 ml 1.0 N NaOH for 60 min at room temperature. After cell lysis, NaOH was neutralized with 0.5 ml 1.0 N HCl. [³H]-GHB uptake into the cells was determined by liquid scintillation counting, and normalized by protein amounts (mg) measured using bicinchoninic acid assay (Pierce, Rockford, IL) with bovine serum albumin as a standard.

Data/Statistical Analysis—Noncompartmental analysis was performed using WinNonlin 5.2 software (Pharsight, Palo Alto, CA) to determine GHB TK parameters. The area under the plasma concentration-time curve (AUC) was determined using the trapezoidal method. Total clearance (CL) was determined as dose/AUC. In all studies, mean values were compared using Student t-test or one-way analysis of variance followed by Tukey's post hoc test for the detection of statistically significant differences in TK/TD parameters. Differences resulting in P-value < 0.05 were considered statistically significant.

Results

Effect of MDMA on the sedative/hypnotic effect of GHB

Co-administration of MDMA with GHB resulted in no significant change in the sedative/hypnotic effect measured as sleep time when compared to the group treated with GHB alone, as presented in Figure 1. MDMA alone did not result in the loss of righting reflex in the animals.

Effect of MDMA on Concentration Dependence of Sedative/Hypnotic Effect of GHB

To evaluate the relationship between GHB concentration and its sedative/hypnotic effect in the presence of MDMA, GHB concentrations were measured in plasma and brain at RRR (offset of sedative/hypnotic effect) in animals treated with GHB alone and with MDMA. GHB plasma and brain concentrations at RRR were not significantly different in the presence of MDMA when compared to animals treated with GHB alone (Table 1), indicating no difference in the concentration-effect relationship.

Effect of MDMA on GHB toxicokinetics

MDMA treatment resulted in no significant changes in the GHB plasma concentration-time profile when compared to GHB alone as shown in Figure 2. The total clearance of GHB was found unchanged in the presence of MDMA (6.53 ± 0.56 ml/min/kg) when compared to GHB alone (6.54 ± 1.02 ml/min/kg). Interestingly, the brain/plasma partitioning of GHB was found to be significantly increased in the presence of MDMA, but there was no statistically significant increase in sleep time (Table 1).

Effect of MCT inhibition on the sedative/hypnotic effect of GHB in the presence of MDMA

Treatment with L-lactate resulted in a significant decrease in GHB-induced sleep time when compared to the GHB alone or GHB-MDMA treatment group, as displayed in Figure 1.

Effect of MCT Inhibition on the Concentration Dependence of Sedative/Hypnotic Effect of GHB

GHB brain and plasma concentrations at RRR were not significantly different in the animals treated with L-lactate when compared to GHB alone or GHB-MDMA, again demonstrating that the concentration-effect relationship of GHB with its sedative/hypnotic effects is maintained after treatment with L-lactate as shown in Table 1. The brain/plasma partitioning of GHB in the presence of L-lactate was found to be significantly lower when compared to the GHB + MDMA group, but was not significantly different when compared to the GHB alone group.

Effect of MDMA on GHB uptake in RBE4 cells

As seen in Figure 3, GHB uptake was significantly increased when compared with GHB alone following a 2 hour pre-incubation with MDMA (0.1, 1 or 10 μ M), which is consistent with our *in vivo* findings.

Discussion

GHB is widely abused as a recreational drug at nightclubs and raves for its euphoric effects. It is commonly co-ingested with other club drugs such as MDMA and ketamine. There is currently no approved treatment strategy for GHB overdose and treatment is limited to supportive care. GHB in a recreational setting is typically not taken alone and is frequently combined with other club drugs and prescription medications³¹. A recent survey of 131 GHB users found that MDMA was co-ingested by 51% of the individuals⁴. Another study in 226 GHB related deaths detected co-intoxications of GHB with CNS depressants such as ketamine and oxycodone in 37%, stimulants such as MDMA in 11%, and both depressants and stimulants in 16.5% of individuals³. MDMA is a widely abused psychostimulant drug that acts by releasing the presynaptic brain neurotransmitters serotonin, dopamine and norepinephrine and also blocking their reuptake^{32,33}. Even though GHB is commonly abused in combination with other drugs in a recreational setting, our knowledge of the effect of co-ingestion of other club drugs on its TK/TD remains limited. Furthermore, potential treatment strategies need to be evaluated under the conditions common to GHB abuse, i.e. the presence of other club drugs. In this study, we assessed the effects of MDMA, a commonly abused club drug, on GHB TK/TD. The effect of MCT inhibition on GHB TK/TD in the presence of MDMA was also evaluated to determine its use as a potential treatment strategy for overdose of GHB when it is co-ingested with MDMA.

Previous results from our laboratory have shown that the sedative/hypnotic effect of GHB increases in a dose-dependent manner and a direct relationship exists between plasma, whole brain, and brain extracellular fluid (ECF) GHB concentration and its sedative/hypnotic effect^{25,29}. Snead *et al.* have also demonstrated that brain GHB concentrations have also been shown to parallel its plasma concentrations and similar relationships with offset of sedative/hypnotic effect have been observed^{34,35}. The results of our study investigating the concentration-effect relationship of GHB with its sedative effects when it is co-administered with MDMA are consistent with previous findings, showing that plasma and brain GHB concentrations remain similar at RRR between the animals treated with GHB in the presence

and absence of MDMA. Even though the brain and plasma concentrations of GHB at RRR were not significantly changed in the presence of MDMA, an interesting finding of this study was a significant increase in the brain/plasma partitioning of GHB at RRR in the presence of MDMA, when compared to the animals treated with GHB alone. In order to further investigate this effect, we studied the effects of MDMA on GHB uptake in RBE4 cells which serve as a good *in vitro* model of the blood brain barrier (BBB). Similar to our *in vivo* results, we found a significant increase in GHB uptake in the presence of MDMA. Our results are also consistent with previous studies looking at the effects of MDMA on butyrate uptake (another MCT substrate) in Caco-2 cells²⁶, where MDMA resulted in a significant increase in butyrate uptake following 20 minutes pre-incubation with MDMA. This requires further investigation since it is possible increased brain uptake might result in significant changes in the concentration-effect relationship at higher doses. Previous reports have shown that GHB is transported across the BBB by MCTs^{24,25} and MCT1 is the only isoform expressed at the BBB³⁶. As the increase in GHB uptake was observed after a short-term pre-incubation of the cells with MDMA (2 hours), we hypothesize that MDMA might affect the short-term regulation of MCTs by affecting its trafficking to the membrane. Molecular mechanisms underlying the short-term regulation of MCTs are poorly understood. Available literature suggests that CD147 (basigin), an ancillary protein, is necessary for trafficking, localization, and functional expression of MCT1 at the plasma membrane, where the two proteins remain tightly bound to each other³⁷. The mechanisms underlying the increased brain uptake may relate to increased trafficking of MCT1 protein to the membrane, post-transcriptional regulation of MCT1 or changes in MCT1-basigin complex expression or stability. However, the factors that contribute to the short-term regulation of MCTs are not completely understood and this needs to be further investigated in future studies. One of the potential treatment strategies that have been evaluated for GHB overdose is use of MCT inhibition. GHB is a substrate for monocarboxylate transporters (MCTs) in the kidney, intestine, and brain^{22–25}. Previous studies have demonstrated that the administration of MCT inhibitors with GHB increases its renal and total clearance in rats due to the inhibition of its active renal reabsorption in the kidney²⁷. In addition, MCT inhibition results in a decrease in the sedative/hypnotic effects of GHB^{27,28}. However, as GHB is not abused alone and is frequently co-ingested with MDMA, it is important to test any proposed treatment strategy for GHB overdose in the presence of MDMA. The results of this study suggest that MCT inhibition can also serve as a detoxifying strategy for GHB when it is co-administered with MDMA, which is consistent with earlier reports on its effect on GHB alone²⁷ or with GHB and alcohol³⁸. The plasma and brain GHB concentrations at RRR after L-lactate administration were not different than GHB alone, similar to previous findings²⁹. Alternatively, GABA_B receptor antagonists have also been proposed for the treatment of GHB overdose. Treatment with GABA_B receptor antagonists has been demonstrated to decrease the sedative/hypnotic effect of GHB³⁹. However, they are currently not approved for use in humans and therefore the use of MCT inhibition with L-lactate offers the advantage of ease of translation to the clinic due to the clinical availability of lactate preparations.

The lack of effect of MDMA on the concentration-effect relationship of GHB with its sedative/hypnotic effect is also further supported by a lack of toxicokinetic interaction

observed in this study. GHB plasma concentration-time profile remained similar when it was co-administered with MDMA. To our knowledge, there is no report in the literature on the effect of MDMA on GHB toxicokinetics and this study serves as a first report indicating a lack of interaction.

In summary, we observe no significant toxicokinetic/toxicodynamic interaction between the club drugs, GHB and MDMA. MDMA co-administration had no significant effect on the sedative/hypnotic effects of GHB. In addition, the concentration-effect relationship of GHB was not affected in the presence of MDMA. However, MDMA can potentially affect the brain/plasma partitioning of GHB, and therefore it remains possible that an in vivo interaction may occur with chronic use or with higher doses. Our results also suggest that MCT inhibition can serve as a potential treatment strategy for GHB overdose when it is co-ingested with MDMA. These results support the use of a therapeutic detoxification strategy that increases GHB elimination which results in a reduction in its sedative/hypnotic effects in an overdose situation where it is combined with MDMA.

Acknowledgments

The authors thank Donna Ruszaj for her assistance in developing the current LC-MS/MS method. This work was supported by the National Institutes of Health National Institute on Drug Abuse [grant DA023223] and by a fellowship from Pfizer Global Research and Development.

Abbreviations

GABA	γ -aminobutyric acid
GHB	γ -hydroxybutyrate
MCT	monocarboxylate transporter
MDMA	3, 4-methylenedioxymethamphetamine
TK/TD	toxicokinetics/toxicodynamics
RRR	return of righting reflex

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Figure 1. Effect of MCT inhibition on the sedative effect of GHB in the presence of MDMA GHB (400 mg/kg i.v.) was administered with or without MDMA (5 mg/kg i.v.). GHB and MDMA were administered with or without L-lactate. L-lactate was administered as 66 mg/kg i.v. bolus plus 302.5 mg/kg/h i.v. infusion 5 minutes after GHB-MDMA administration and continued until animals were euthanized at RRR. Sleep time was measured as the difference between the loss and return of righting reflex. One-way analysis of variance followed by Tukey's post-hoc test was used to determine statistically significant differences in sleep time between different treatment groups. Data presented as mean $\pm$ SD, $n=3-5$. * $P < 0.05$ significantly different from GHB alone or GHB + MDMA.

Figure 2. Plasma concentration-time profiles of GHB (400 mg/kg i.v.) in the presence and absence of MDMA
MDMA was administered as 5 mg/kg i.v. bolus. Data are presented as mean $\pm$ S.D. (n = 4).

Figure 3. Effect of MDMA on the uptake of GHB in RBE4 cells

Cells were pre-incubated with MDMA (0.1, 1, or 10 μ M) at 37°C for 2 h. Data presented as mean $\pm$ SD of three sets of studies conducted in triplicate. One-way analysis of variance followed by Tukey's post-hoc test was used to determine statistically significant differences between different treatment groups. * $P < 0.05$ significantly different from GHB alone.

Table 1
Effect of MDMA on plasma and brain GHB concentrations at RRR in the presence and absence of L-lactate

GHB (400 mg/kg i.v.) and MDMA (5 mg/kg i.v.) were administered with or without L-lactate. L-lactate was administered as 66 mg/kg i.v. bolus plus 302.5 mg/kg/h i.v. infusion 5 minutes after GHB-MDMA administration and continued until RRR. Animals were euthanized at RRR and brain and plasma samples collected.

	C _{plasma} GHB (µg/ml)	C _{brain} GHB(µg/g)	GHB brain/plasma ratio
GHB alone	348 ± 29.3	73.0 ± 10.2	0.18 ± 0.02
GHB + MDMA	321 ± 40.0	78.1 ± 4.33	0.25 ± 0.03 *
GHB + MDMA + L-lactate	419 ± 70.1	75.2 ± 6.00	0.18 ± 0.03 [‡]

Data presented as mean ± SD, n=3–5. One way analysis of variance followed by Tukey's post-hoc test was used to determine statistically significant differences between treatment groups.

* P < 0.05 significantly different from GHB alone group;

[‡] P < 0.05 significantly different from GHB + MDMA group.