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Endogenous concentrations, pharmacokinetics, and selected pharmacodynamic effects of a single dose of exogenous GABA in horses

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The anti-anxiety and calming effects following activation of the GABA receptor have been exploited in performance horses by administering products containing GABA. The primary goal of the study reported here was to describe endogenous concentrations of GABA in horses and the pharmacokinetics, selected pharmacodynamic effects, and CSF concentrations following administration of a GABA-containing product. The mean (±SD) endogenous GABA level was 36.4 ± 12.5 ng/mL (n = 147). Sixteen of these horses received a single intravenous and oral dose of GABA (1650 mg). Blood, urine, and cerebrospinal fluid (n = 2) samples were collected at time 0 and at various times for up to 48 h and analyzed using LC-MS. Plasma clearance and volume of distribution was 155.6 and 147.6 L/h and 0.154 and 7.39 L for the central and peripheral compartments, respectively. Terminal elimination half-life was 22.1 (intravenous) and 25.1 (oral) min. Oral bioavailability was 9.81%. Urine GABA concentrations peaked rapidly returning to baseline levels by 3 h. Horses appeared behaviorally unaffected following oral administration, while sedative-like changes following intravenous administration were transient. Heart rate was increased for 1 h postintravenous administration, and gastrointestinal sounds decreased for approximately 30 min following both intravenous and oral administration. Based on a limited number of horses and time points, exogenously administered GABA does not appear to enter the CSF to an appreciable extent.

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INTRODUCTION

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) (Roberts & Kuriyama, 1968). It is found in high concentrations in the CNS, where it is synthesized from the excitatory neurotransmitter, glutamate (Foster & Kemp, 2006). Because of its inhibitory actions, agonists that bind to the GABA receptor have been exploited for interventional procedures requiring sedation or anesthesia (Petroff, 2002) as well as developed for a number of medical conditions where excitatory neurotransmission predominates (Kuriyama *et al.*, 1966; Simler *et al.*, 1973; Jung *et al.*, 1977; Rando, 1977; Atack, 2005; Foster & Kemp, 2006).

The anti-anxiety and calming effects following activation of the GABA receptor have been exploited in performance horses by synthesizing and administering products, such as Carolina Gold and other compounded formulations, containing GABA. As the potential detrimental effects are unknown and because of its potential to enhance performance, products containing GABA have been added to the banned substances list by both the United States Equestrian Federation and the Federation Equestre Internationale. Regulation of this compound has proven to be challenging, primarily because as a naturally occurring substance, differentiating between exogenously administered GABA and naturally occurring GABA is not possible using conventional liquid chromatography-mass spectrometry (LC-MS) analyses. Adding to the challenge of regulating this substance is the likelihood of varying endogenous concentrations between horses of different breeds and ages.

The administration of GABA to horses prior to performance for its purported 'calming effect' necessitates further study of

this substance so it can be properly regulated. The purpose of the current study was to (i) describe baseline concentrations of GABA in horses of varying breeds and ages, (ii) describe the pharmacokinetics of exogenous GABA following intravenous and oral administration, (iii) describe the behavioral and selected physiologic effects of exogenous GABA administration, and (iv) describe GABA concentrations in cerebrospinal fluid prior to and following intravenous GABA administration.

MATERIALS AND METHODS

Baseline GABA determinations

For baseline plasma GABA determinations, a total of 147 horses were studied. Horses studied included University owned research horses and horses housed at three local training/boarding facilities within a 70-mile radius. Horses included 97 Thoroughbreds (horses in race training, broodmares and University owned research horses), 16 Quarter Horses (pleasure show horses and University owned research horses), nine Paint Horses (pleasure show horses), four Warmbloods (3-day Eventing horses), four Hanoverians (3-day Eventing horses), six Irish Sport Horses (3-day Eventing Horses and University owned research horses), six Arabians (University owned research horses), one Westphalian (University owned research horse), one pony (University owned research horse), one Lusitano (University owned research horse), one Oldenburg (3-Day eventing horse), and one Holsteiner (3-day Eventing horse). Plasma samples were collected on five different occasions over a 2-week period from 16 horses in the exercised Thoroughbred group (see below) to assess variability in GABA concentrations on different sampling occasions and at different times during the day. A single blood sample was collected from the remaining horses. For all horses, samples were collected by direct venipuncture into EDTA blood tubes, followed by centrifugation at 3000 ***g*** for 10 min, immediate transfer of plasma into storage cryovials and storage at −20 °C until analyzed. The breed, age, and gender of each horse were recorded at the time of sample collection. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

GABA administration studies

Animals. For the drug administration study, sixteen University owned healthy exercised to racing fitness adult Thoroughbred horses including eight geldings and eight mares (4–7 years of age; mean ± SD weight of 535 ± 33 kg) were studied. Prior to and throughout the course of the study, horses were exercised 5 days a week, following standard protocols established by our laboratory. The general exercise protocol for these horses consists of 3 days per week on an Equineciser (Centaur Horse Walkers Inc, Mira Loma, CA, USA) (5-min walk; 20-min trot; 5-min walk) and 2 days per week on a high-speed treadmill (Mustang 2200; Graber AG, Fahrwangen, Switzerland) (5 min @ 1.9 m/sec; trot 5 min @ 4 m/sec; canter 2 min @ 10 m/sec; walk 5 min @ 1.9 m/sec or 5 min @ 1.9 m/sec; trot 5 min @ 4 m/sec at 4 degree incline; walk 5 min @ 1.9 m/sec).

Before beginning the study, horses were determined healthy and free of disease by physical examination, complete blood count and a serum biochemistry panel that included aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, total bilirubin, sorbitol dehydrogenase, blood urea nitrogen, and creatinine. Blood analyses were performed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using standard their protocols. Horses did not receive any other medications for at least 2 weeks prior to commencement of this study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

Instrumentation and drug administration. Throughout the course of the study, horses were housed in box stalls at the Veterinary Medicine Teaching Hospital. Stalls used for this study were located as far as possible from treatment rooms and receiving areas of the hospital. Drug administration commenced at 6:00 am to minimize, as much as possible, the number of external distractions during the collection of physiologic measurements and behavioral observations. This study was conducted in a randomized, balanced two-way crossover design, wherein eight horses received a single intravenous administration of 10 mL of an injectable formulation containing 165 mg/mL GABA and eight horses received a single administration of 10 mL of a similar oral formulation during the first administration. Following a washout period of 2 weeks, the drug administration scheme was switched. The dose for both formulations was chosen based on an informal verbal survey of competitors at horse shows as well as hunter-jumper and 3-day Eventing competitions. As there is currently no commercially available FDA-approved injectable or oral formulations containing GABA, the products used in the current study were purchased from a compounding pharmacy. The actual concentration of each formulation was measured each week prior to administration, as described in the Sample Analysis section below. Food was withheld for 12 h prior and 4 h postdrug administration only for the oral administration. Water was available *ad libitum* throughout the duration of the study.

A14-gauge catheter was aseptically placed in each external jugular vein for horses receiving the intravenous formulation. The right jugular vein catheter was used for drug administration, while the contralateral catheter was used for sample collection. The right jugular vein catheter was removed following dosing. A single 14-gauge jugular vein catheter was placed for horses receiving the oral formulation.

Sample collection. Blood. Blood samples were collected at time 0 (prior to drug administration) and at 5, 10 (intravenous administration only), 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 36, and 48 h postdrug

administration. Prior to drawing each sample of blood for analysis of drug concentrations, 10 mL of blood was aspirated and discarded from the catheter and T-Port extension set (combined internal volume <2 mL). The catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL) following each sampling time. The jugular vein catheter, used for sample collection, was removed following the 18-h sample collection and the remaining samples collected via direct venipuncture. Blood samples were collected into EDTA blood tubes (Kendall/Tyco Healthcare, Mansfield, MA, USA) and stored on ice until centrifugation at 1620 ***g*** for 10 min at 4 °C. Plasma was then immediately transferred into storage cryovials (Phenix Research Products, Chandler, NC, USA) and stored at −20 °C until analysis (approximately 2 weeks following collection of the final sample).

Urine. Urine samples were collected from four mares administered GABA intravenous. Prior to drug administration, a Foley urinary catheter was placed using sterile technique. Samples were collected at 1, 3, 4, 6, and 24 h postdrug administration. The urinary catheter was removed following the 6-h sample collection and the 24-h sample collected via free catch. Urine samples were stored at −20 °C (approximately 2 weeks) until analysis.

Cerebrospinal Fluid. Although not necessarily indicative of actual brain levels, cerebrospinal fluid samples were collected following exogenous administration of GABA to assess whether GABA was capable of crossing from the blood into the CSF and thereby whether the potential existed for movement into brain tissue. Cerebrospinal fluid and corresponding blood samples were collected from two horses dosed with intravenous GABA. Samples were collected immediately prior to GABA administration and at 15 (two horses), 30 (one horse), and 45 min (one horse) postdrug administration. Prior to collection of the baseline sample and GABA administration, horses were anesthetized with 1.1 mg/kg xylazine (Lloyd Inc, Shenanodoah, IA, USA) intravenous, 2 mg/kg ketamine (Zoetis, Florham Park, NJ, USA) intravenous, and 0.008 mg/kg butorphanol tartrate (Torbugesic, Zoetis) intravenous. Additional xylazine (100–150 mg intravenous) or ketamine (200 mg intravenous) was administered as necessary throughout the collection period. Once the horse was laterally recumbent, the poll area was clipped, aseptically prepared and the skin blocked with 0.5 mL of 2% lidocaine. The atlantooccipital subarachnoid space was identified and a spinal needle inserted into the space. Approximately 1.5–2 mL of cerebrospinal fluid was withdrawn at each time point. Following collection of the baseline sample, horses were dosed intravenous with 10 mL of a compounded product containing 165 mg/mL of GABA.

Determination of GABA concentrations. The analytical reference standards for 4-aminobutyric acid (GABA) and the internal standard D3-methionine were bought as a 200 nmol/mL solution from Phenomenex (Torrance, CA, USA). Water was purchased from Burdick and Jackson (Muskegon, MI, USA). Methanol and ammonium formate were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). The solvents were HPLC grade or better.

The 4-aminobutyric acid (GABA) working solution was prepared by dilution of the 200 nmol/mL stock solution with water to concentrations of 0.1, 1, 10, and 100 pmol/μL. Calibrators were prepared at concentrations of 0.01, 0.1, 0.5, 1, 5, 10, 50, 100, 150, and 200 nmol/mL. Calibration curves were prepared fresh for each quantitative assay. In addition, quality control samples, at three concentrations within the standard curve, were included with each sample set as an additional check of accuracy. Calibrators and quality control samples did not have control plasma added due to the presence of endogenous GABA.

Prior to analysis, samples were extracted and derivatized with EZ:faast-Free (Physiological) Amino Acid Analysis kit purchased from Phenomenex. Samples were prepared according to the manufacturer's instructions. Samples were reconstituted in 100 μL of 1:2 (V:V) water:methanol, both with 10 mM ammonium formate, and 10 μL was injected into the LC-MS/MS system.

The concentration of 4-aminobutyric acid (GABA) and the internal standard D3-methionine were measured in plasma, serum, urine, CSF, and dosing solution by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using positive heated electrospray ionization (HESI(+)). Quantitative analysis of plasma, serum, urine, CSF, and dosing solution was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled with a turbulent flow chromatography system (TFC TLX4 Thermo Scientific, Franklin, MA, USA) having 1100 series liquid chromatography systems (Agilent Technologies, Palo Alto, CA, USA) and operated in laminar flow mode. The spray voltage was 3030V, the vaporizer temperature was 301 °C, and the sheath and auxiliary gas (nitrogen) were 40 and 20, respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the standards into the TSQ Vantage. Chromatography employed an EZ:faast 4u AAA:MS 250 × 2 mm column (Phenomenex) and a linear gradient of methanol in water with a constant 10 mM ammonium formate, at a flow rate of 0.25 mL/min. The initial methanol concentration was held at 65% for 0.33 min, ramped to 90% over 8.34 min, before re-equilibrating for 4.5 min at initial conditions.

Detection and quantification was conducted using Selective Reaction Monitoring (SRM) of initial precursor ion for GABA (mass to charge ratio 232.19 (m/z)) and the internal standard D3-methionine (mass to charge ratio 281 m/z). The response for the product ions for GABA (86, 130, 172 m/z) and the internal standard D3-Methionine (193, 221 m/z) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate GABA in all samples. A weighting factor of 1/X was used for all calibration curves.

A linear calibration curve was used for GABA and gave a correlation coefficient (R^2) of 0.99 or better. The intraday, in-

terday, analyst-to-analyst precision, and accuracy of the assay were determined by assaying quality control samples in replicates ($n = 6$) for GABA. Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation (Table 1). The technique was optimized to provide a limit of quantitation of 0.1 for GABA.

Pharmacokinetic calculations. Plasma concentration data were modeled in Phoenix WinNonlin 6.3 (Certera, Cary, NC, USA) by adding a baseline (endogenous concentrations) correction to a conventional 2-compartment population model. Endogenous levels were determined by averaging GABA concentrations collected on two separate occasions (3 days and immediately prior to exogenous GABA administration). C_{max} and time to maximal plasma concentration (T_{max}) were determined directly from the plasma concentration data. The elimination rate constant was calculated by determination of the slope of the terminal portion of the plasma concentration vs. time curve and the plasma elimination half-life using the formula (ln 2)/the elimination rate constant. The AUC_{last} was calculated using the log up/linear down trapezoidal method. Bioavailability of the oral formulation was calculated using the formula (AUC_{PO}/AUC_{IV}) * ($dose_{IV}/dose_{PO}$). Pharmacokinetic parameters for GABA are reported as mean values.

Physiological responses and behavioral monitoring. Horses were unrestrained for the duration of the study and were only restrained, if necessary, for sample collection and physiologic parameter collection. Behavioral assessments were made from outside the stall and all physiologic parameters were collected immediately prior to blood sample collection. Seven of the horses in the intravenous group were equipped with two Step Monitors (SAMe, Seattle, WA, USA) programmed to count the number of steps taken each minute. Step counters were calibrated the day prior to commencement of the study by manually counting the number of steps taken over a 2-min period by a nontreated horses (total of six horses) and comparing this number to the number of steps recorded by the Step Monitors. This process was repeated six times per horse. The accuracy, calculated as the percent nominal value, was 97.8%. Monitors were fastened by a Velcro strap to the lateral side of the left lower front and hind leg of each horse. The distal portion of all four legs were wrapped with polo wraps, to minimize favoring of one leg over another. The number of steps was recorded continuously for a minimum of 30 min prior to and 4 h postdrug administration. Chin to ground distance, gastrointestinal (GI) borborygmi, and other behavioral observations were made by the same individual, blinded to the treatment of the animal, at all time points. Chin to ground distance was also monitored for all horses prior to and for 4 h postdrug administration. The distance from the animal's muzzle to the ground was measured using a measuring tape. GI borborygmi were monitored by direct auscultation at 0 (immediately prior to drug administration) and 15, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h post-GABA administration. The upper and lower flank area on the left and right side were auscultated and a numerical score equal to the number of borborygmi per 30 sec in each quadrant was assigned. A score of 0.5 was added for uncoordinated rumbling or gaseous sounds for each quadrant. Scores from the four quadrants were summed to provide a single GI motility score. Additional notable physiologic or behavioral observations were recorded throughout the course of the study.

Horses were also equipped with a Holter monitor (Forrest Medical, East Syracuse, NY, USA) to assess any potential effect on heart rate. Heart rate was recorded continuously for a minimum of 30 min pre- and 4 h postdrug administration. Heart rate was determined at predetermined time points via manual counting of P-QRS-T complexes over a 1-min time period.

Statistical analysis

Statistical analyses using commercially available software (SAS, Cary, NC, USA) were performed to assess significant differences in physiologic variables for individual horses following intravenous and oral GABA administration. Raw data for all variables were checked for normality using the Wilk–Shapiro test and then log-transformed or Winsorized as necessary to bring the residual distribution in close agreement with a normal distribution. Data for all variables were subsequently analyzed using a mixed model ANOVA with repeated measures. Significance was set at $P < 0.05$.

RESULTS

Baseline concentrations, pharmacokinetic analysis, and urinary concentrations

Naturally occurring (endogenous) GABA concentrations for the different breeds and ages studied are listed in Table 2A, B. The mean (±SD) endogenous GABA concentration for all 147 horses was 36.4 ± 12.5 ng/mL. Endogenous GABA concentra-

Table 1. Accuracy and precision values for LC-MS/MS analysis of GABA in equine plasma

Concentration (ng/mL)	Intraday accuracy (% nominal concentration)	Intraday precision (% relative SD)	Interday accuracy (% nominal concentration)	Interday precision (% relative SD)
30.9	110.0	20.0	108.0	16.0
4124.8	97.0	9.0	103.0	8.0
18561.0	98.0	15.0	102.0	13.0

Table 2. Endogenous GABA plasma concentrations for different breeds of horses at varying ages

A

			[GABA] ng/mL		
Age (years)	Breed	Number	Mean ± SD	Median	Range
0–5	TB	28	40.0 ± 9.6	38.1	24.7–68.8
	QH	4	66.5 ± 12.5	70.1	48.5–77.3
	Paint	–	–	–	–
	WB	1	48.5 ± 0.0	NA	NA
	Hanoverian	1	49.5 ± 0.0	NA	NA
	Irish Sport Horse	–	–	–	–
	Arabian	–	–	–	–
6–10	TB	33	37.5 ± 12.6	38.6	12.9–55.0
	QH	3	50.8 ± 6.5	47.7	46.4–58.2
	Paint	4	48.7 ± 18.4	46.8	28.9–72.4
	WB	1	41.2 ± 0.0	NA	NA
	Hanoverian	1	40.2 ± 0.0	NA	NA
	Irish Sport Horse	4	46.9 ± 6.1	49.0	38.2–51.6
	Arabian	–	–	–	–
11–15	TB	17	30.4 ± 8.2	29.1	16.2–51.2
	QH	1	21.2 ± 0.0	NA	NA
	Paint	4	33.0 ± 16.3	37.7	9.6–46.9
	WB	1	36.1 ± 0.0	NA	NA
	Hanoverian	1	30.9 ± 0.0	NA	NA
	Irish Sport Horse	2	60.6 ± 10.5	60.6	53.2–68.1
	Arabian	–	–	–	–
16–20	TB	13	32.0 ± 17.6	27.1	15.9–63.9
	QH	2	41.8 ± 4.5	41.8	38.6–45.0
	Paint	1	35.1 ± 0.0	NA	NA
	WB	–	–	–	–
	Hanoverian	–	–	–	–
	Irish Sport Horse	–	–	–	–
	Arabian	1	24.3 ± 0.0	NA	NA
20–25	TB	6	31.9 ± 4.2	34.0	26.0–35.1
	QH	6	32.0 ± 5.2	30.2	26.5–41.5
	Paint	–	–	–	–
	WB	1	29.5 ± 0.0	NA	NA
	Hanoverian	1	28.9 ± 0.0	NA	NA
	Irish Sport Horse	–	–	–	–
	Arabian	5	38.0 ± 7.7	37.0	26.6–47.4

B

Breed	Age (year)	Number	[GABA] (ng/mL)
Westphalian	11	1	55.7
Pony	18	1	23.9
Lusitano	7	1	57.7
Oldenburg	8	1	39.2
Holsteiner	6	1	24.7

tions for 16 horses sampled on five different occasions are listed in Table 3.

The compounded GABA solutions were tested for purity and potency prior to each of the administrations. All formulations were found to be within 10% of the nominal concentration prior to each administration. Average GABA plasma concentration vs. time curves for the injectable and oral formulations are depicted in Fig. 1. Compartmental analysis with modification of the model to incorporate endogenous (nonzero) baseline values was used to analyze plasma concentration data. Selected pharmacokinetic parameters for exogenous GABA administration are listed in Table 4. The volume of the central compartment (V_1) was smaller than the volume of the peripheral compartment (V_2). Absorption was poor following oral administration (F_{PO} = 9.81%). The elimination half-life was short (intravenous: 22.1 min; PO: 25.1 min) and plasma clearance rapid.

Average urine concentrations peaked rapidly following intravenous administration (Fig. 2) with the highest concentration measured at the first time point sampled (1 h postadministra-

Table 3. Endogenous GABA plasma concentrations from 16 exercised Thoroughbred horses following collection on five different occasions over a 2-week period

	[GABA] (ng/mL)					
Horse #	Sample 1 (pm)	Sample 2 (am)	Sample 3 (pm)	Sample 4 (am)	Sample 5 (pm)	Mean ± SD
1	39.9	38.4	43.8	41.4	65.6	45.8 ± 11.2
2	52.2	31.6	29.0	42.6	43.7	39.8 ± 9.5
3	54.8	45.1	74.9	49.8	51.2	55.2 ± 11.6
4	41.8	31.8	34.9	31.8	34.9	35.0 ± 4.1
5	43.8	28.9	53.2	47.7	37.5	42.2 ± 9.4
6	37.4	61.0	64.7	61.1	57.6	56.4 ± 10.9
7	41.9	34.9	40.1	34.5	38.6	38.0 ± 3.2
8	38.6	36.4	40.3	24.9	35.6	35.2 ± 6.0
9	48.9	42.2	82.7	45.7	45.1	52.9 ± 16.8
10	68.8	36.8	68.0	51.7	53.7	55.8 ± 13.2
11	34.9	36.7	55.7	46.9	35.0	41.8 ± 9.2
12	26.1	42.2	47.1	25.1	41.7	36.4 ± 10.1
13	53.6	43.9	53.0	45.0	49.5	49.0 ± 4.5
14	53.9	35.7	59.9	47.6	42.7	48.0 ± 9.4
15	55.0	49.4	34.3	51.6	51.3	48.3 ± 8.1
16	50.1	39.1	47.3	38.3	45.0	44.0 ± 5.1

am, morning sample collection; pm, evening sample collection.

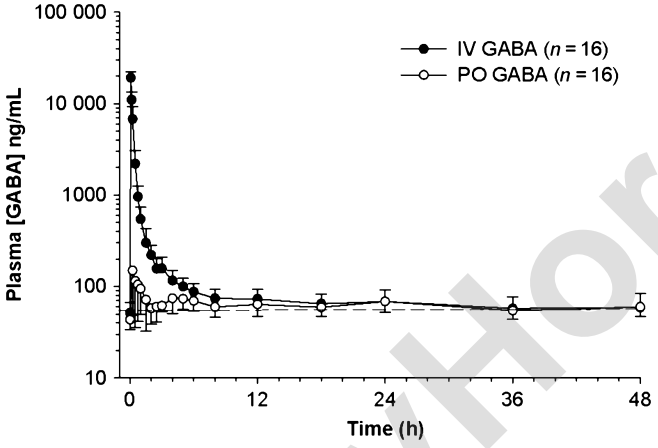

Fig. 1. Mean plasma concentration vs. time curves following a single intravenous or administration of 1650 mg of GABA to 16 exercised Thoroughbred horses. Data are expressed as mean ± SD.

Table 4. Selected pharmacokinetic parameters (mean) following intravenous and oral administration of 1650 mg of GABA to 16 exercised thoroughbred horses

	Intravenous	Oral
K_{el} (1/h)	1.88	1.65
K_{el} HL (h)	0.368	0.419
AUC (h*mg/L)	10.6	1.04
V_1 (L)	0.154	–
V_2 (L)	7.39	–
Cl_1 (L/h)	155.6	–
Cl_2 (L/h)	147.6	–
F (%)	–	9.81

K_{el}, terminal elimination rate constant; K_{el} HL, terminal half-life; AUC, area under the plasma concentration–time curve; V_1, volume of the central compartment; V_2, volume of the peripheral compartment; Cl_1, clearance from the central compartment; Cl_2, clearance from the peripheral compartment; F, bioavailability.

tion) and concentrations returning to baseline levels by 3 h postdrug administration.

GABA CSF concentrations with corresponding plasma concentrations for two horses at three time points are listed in Table 5. Prior to GABA administration, concentrations in plasma and CSF were comparable in both horses. In the one horse sampled, GABA plasma concentrations were nearly 46 times higher in plasma as compared to CSF at 15 min postadministration. At 30 min post-GABA administration, CSF concentrations were approximately twice as high as baseline levels but still approximately 46 times lower than plasma concentrations in horse 1. For horse 2, CSF concentrations were the same as baseline concentrations at 30 min post-GABA administration but over 100 times lower than plasma concentrations. At the last time point sampled (45 min), CSF concentrations remained the same as baseline concentrations in horse 2 and were 33 times lower than plasma concentrations.

Behavioral and physiological responses

Within 0.5–1 min of intravenous administration of GABA, 10/16 horses exhibited a 'bowing' posture, shifting their weight to their hind end and stretching their front legs out in front of them. This was followed by extension of their hind legs. Horses (14/16) also curled their upper lip. One horse appeared to want to lie down 2 min postdrug administration. These responses were short-lived with horses returning to regular behavior (eating and moving about the stall) within 5 min of administration. No behavioral changes, including signs of sedation, were noted following oral administration at any time

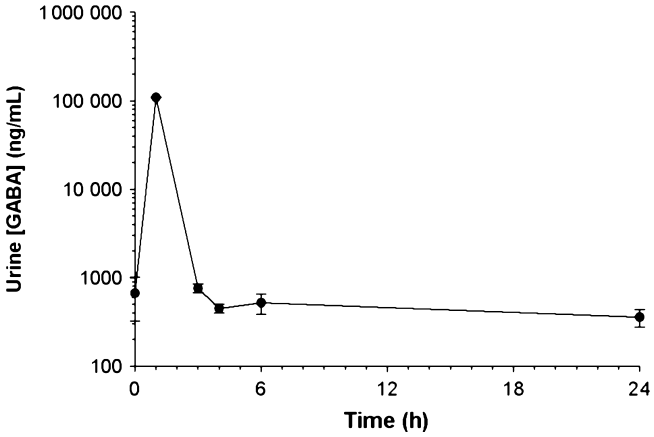

Fig. 2. Mean urine concentration vs. time curve following a single intravenous administration of 1650 mg of GABA to four horses. Data are expressed as mean ± SD.

postadministration. The average number of steps taken by the horses following administration of GABA are depicted in Fig. 3a,b for intravenous and oral administration, respectively. Although not statistically significant, the average number of steps increased above pretreatment values for the first 10 min postdrug administration for both routes of administration. By 30 min post-GABA administration, the number of steps for all horses receiving either formulation decreased to baseline values. There was no significant difference in chin to ground distance relative to baseline following either oral or intravenous GABA administration (Fig. 4).

Average changes in heart rate for intravenous and oral administration of GABA are depicted in Fig. 5. Heart rate did not change significantly following oral GABA administration; however, following intravenous administration, the heart rate increased significantly with maximum changes noted at 2 min postadministration. The heart rate, following intravenous administration, remained above baseline values until approximately 1 h post-GABA administration.

Gastrointestinal (GI) sounds were decreased by 15 min post-GABA administration following both oral and intravenous administration (Fig. 6), increasing toward baseline levels starting 30 min postadministration. Feces were noted in the stalls of all horses throughout the observation period with fecal consistency normal throughout the study for all horses.

DISCUSSION

Administration of exogenous GABA to performance horses for its purported sedative-like effects has led us to investigate the PK/PD effects of this compound when administered to horses. Additionally, as GABA is an endogenous substance, a secondary goal of this study was to assess the feasibility of regulating the use of exogenous GABA in performance horses, including comparison of GABA concentrations in horses of different breeds and comparing concentrations following repeated measures at different points in time from the same horse.

Samples were collected from a number of horses to assess variability in endogenous GABA concentrations between breeds and different age groups as well as within a single horse at different times. Based on findings from the current study, there appears to be a great deal of variability in GABA plasma concentrations between horses, but no trends with respect to breed or age could be established. For assessment of variability in GABA concentrations within an individual horse, samples were collected from the same horses at five different points in time over the course of 2-weeks. While this method of assessment does not lend itself to statistical analysis of concentrations from individual horses at the different collection times, there are obvious disparities in concentrations between samples collected on different occasions from the same animal that is beyond analysis variability. This suggests that determining a normal threshold concentration may require incorporation of a safety factor to account for this variability. Interestingly, however, the variability noted at the different sampling times does not appear to be related to the time of day at which the samples were collected (am or pm).

GABA plasma concentrations rapidly exceeded baseline levels following intravenous administration; however, following oral administration exogenous GABA was poorly absorbed with only moderate increases in GABA plasma concentrations above pre-administration (baseline) values. Reports describing the disposition of GABA following exogenous administration are limited (Van Gelder & Elliot, 1958; Kuriyama & Sze, 1971; Toth & Lajtha, 1981; Vignolo *et al.*, 1992). In one study, GABA was rapidly absorbed and widely distributed following IP administration to rats, with measurable concentrations readily achieved in kidney, liver, and muscle (Van Gelder & Elliot, 1958). Conversely, nonsignificant changes in brain GABA concentrations were noted following exogenous administration (Van Gelder & Elliot, 1958). In the same study,

Table 5. Cerebrospinal fluid GABA concentrations in two horses following intravenous administration of 1650 mg of GABA to two horses

	Horse 1		Horse 2	
Time (min)	[Plasma] (ng/mL)	[CSF] (ng/mL)	[Plasma] (ng/mL)	[CSF] (ng/mL)
Baseline	39.2	53.6	43.3	48.5
15	8091	175.3	–	–
30	4571	99.0	5189	48.5
45	–	–	1433	43.3

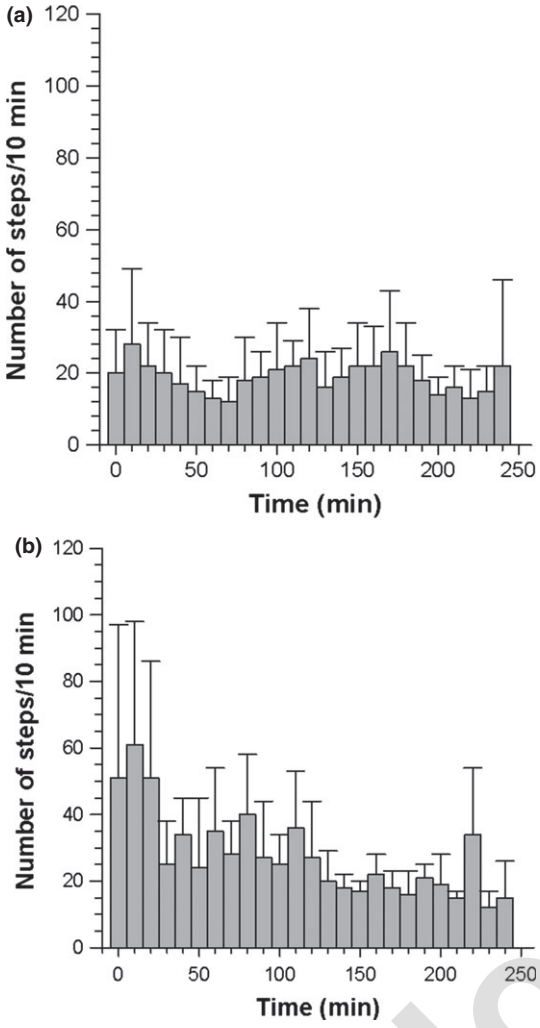

Fig. 3. Number of steps taken with respect to time following intravenous (a) and (b) administration of 1650 mg of GABA to 16 exercised Thoroughbred horses. The number of steps recorded from the front and hind legs from individual horses were averaged at each time point and the number of steps summed over 10 min increments. Data are expressed as mean ± SD.

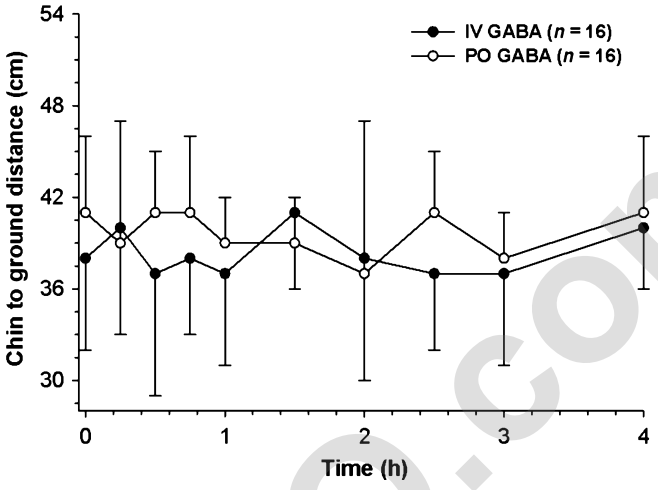

Fig. 4. Chin to ground distance with respect to time following intravenous and oral administration of 1650 mg of GABA to 16 exercised Thoroughbred horses. Data are expressed as mean ± SD.

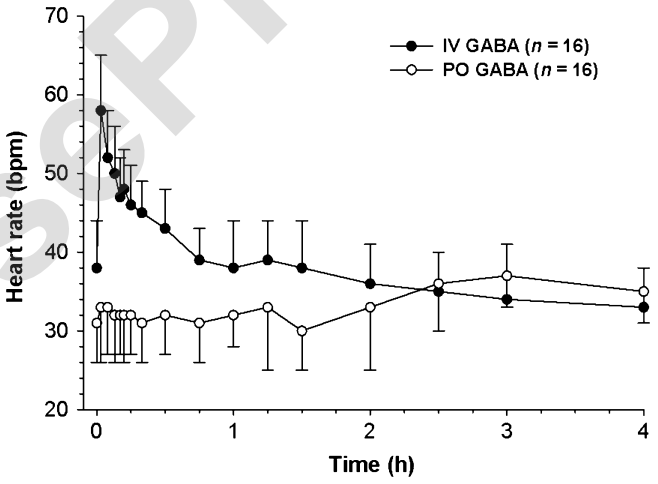

Fig. 5. Heart rate with respect to time following intravenous and oral administration of 1650 mg of GABA to 16 exercised Thoroughbred horses. Data are expressed as mean ± SD.

investigators reported low levels of GABA (0.2 μmols/mL) in CSF following intravenous administration to a cat. While brain tissue from the cat was not examined, lack of detection of GABA in the brain of rats coupled with detection of low levels of GABA in the CSF of cats led the authors to suggest that the blood-CSF barrier effectively prevents any appreciable movement of exogenous GABA into the brain (Van Gelder & Elliot, 1958). In another report, investigators reported an increase in GABA levels in the brain following oral administration of very large doses of GABA (resultant plasma concentrations of 7–50 mM). The lack of reliability of drug concentrations in the CSF as a predictor of brain concentrations has since been described (Bonati *et al.*, 1982; de Lange & Danhof, 2002; Pardridge, 2011). Many drugs are able to cross the microvascular endothelium of the BBB but are unable to subsequently cross the blood-CSF barrier. In particular, the relationship between CSF and brain GABA levels following exogenous administration is not clear. Kuriyama and Sze (1971) determined that radiolabeled GABA did not enter the brain following IP administration. Conversely, other investigators have demonstrated that GABA when administered at high doses accumulates in brain tissue (Bohlen *et al.*, 1979; Vignolo *et al.*, 1992). While it was not possible to measure GABA concentrations in the brain in the current study, CSF concentrations were quantified. Although CSF concentrations of a drug are not necessarily a good predictor of brain levels, because drug must pass through the CSF to enter the brain, the minimal (horse 1) to absent (horse 2) increase in GABA levels in the CSF following intravenous administration, suggests that the likelihood of GABA reaching the brain following systemic administration in horses is minimal. An admitted shortcoming of this study is that CSF concentrations were only measured in two horses and only a very limited number of samples

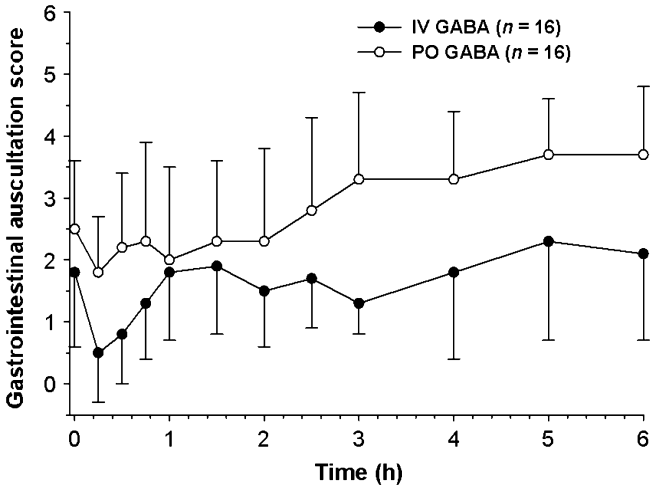

Fig. 6. Gastrointestinal motility score following intravenous and oral administration of 1650 mg of GABA to 16 exercised Thoroughbred horses. Data are expressed as mean ± SD.

post-GABA administration collected. Therefore, it should be noted that the data in the presently reported study is very much limited and as such the comments made here as to the ability, or lack thereof, of exogenous GABA to reach brain tissue are only speculative. As this was only a preliminary assessment of concentrations in the CSF following exogenous GABA administration, with a limited number of sample collections within 45 min, the protocol selected for CSF collection was to anesthetize the animals and collect samples by means of a spinal needle at each time point. A more complete picture of CSF concentrations post-GABA administration could be determined through the use a previously implanted spinal catheter. This would allow for the collection of samples for a longer duration of time and potentially eliminate the necessity for anesthesia during sample collection. The sedatives used in the current study (xylazine, ketamine, and butrophanol) were chosen in part because they are not known to act via modulation of GABA levels; however, eliminating the need to use anesthesia for this experiment would certainly be more desirable as the effect of anesthesia on GABA concentrations has not been described.

Plasma clearance of exogenous GABA appears to be rapid, with concentrations declining quickly following both intravenous and oral administration. The elimination half-life for GABA in the current study was determined to be 22.1 and 25.1 min for intravenous and oral administration, respectively, which is in close agreement to the 20 min reported for rabbits following IP administration and cats following intravenous administration (Van Gelder & Elliot, 1958). To the authors knowledge, there are no other reports describing the elimination half-life of exogenous GABA in any species.

The use of GABA in performance horses is related to its reported sedative-like effects. While horses appeared behaviorally affected (bowing and stretching) immediately following intravenous GABA administration, the effects were short-lived (<5 min). While the mechanism for this response cannot be determined from the current study, due to the immediacy of the reaction, we hypothesize that this effect is likely not centrally mediated. Coinciding with this behavioral effect was a statistically significant increase in heart rate (relative to baseline) that lasted for 15 min postintravenous administration. While the increase in heart rate following administration of an inhibitory neurotransmitter such as GABA, may seem counter intuitive, GABA administration to conscious animals has been similarly shown to result in an increase in heart rate (Bentzen & Grunnet, 2011). The proposed mechanism for this GABA-induced tachycardia is via inhibition of preganglionic cardiac vagal neurons followed by a subsequent reduction in muscarinic acetylcholine receptor activity (Bentzen & Grunnet, 2011). A modest time-related increase in heart rate following oral administration of GABA was also noted starting at 2.5 h postadministration. Due to the long lag time in effect and the low plasma concentrations at this time, it is likely that this increase is most likely due to environmental stimulation and not a result of drug administration.

$GABA_B$, one subclass of GABA receptors, is abundantly expressed in the GI tract (Hyland & Cryan, 2010). To assess the effects of exogenous GABA administration on GI motility, we monitored GI borborygmi for a period of time post-GABA administration. GI auscultation scores were significantly reduced compared with baseline at 15 min postadministration following both oral and intravenous administration. This supports previous reports of inhibition of excitatory acetylcholine release in the small GI tract, leading to reduced GI motility (Hyland & Cryan, 2010). This reduction of GI motility was short-lived, however, with motility scores returning to baseline levels by 30 and 45 min postadministration for intravenous and oral administration, respectively.

In summary, this study sought to characterize endogenous concentrations in a variety of breeds as well as to describe plasma, urine, and CSF concentrations and the pharmacokinetics of GABA following exogenous administration. Additionally, observations of select pharmacodynamics actions, including behavioral and physiologic effects were assessed. Results of the current study demonstrate a large degree of variability between horses with respect to endogenous GABA concentrations. Nonetheless, exogenous administration of GABA elicited a significant rise in Thoroughbred horse plasma GABA concentrations following intravenous administration. Following oral administration, GABA plasma concentrations increased only minimally over baseline levels. Lastly, based on the single dose administered in the current study, GABA administration, elicited a transient and minimal degree of sedation with a concurrent increase in heart rate postintravenous administration.

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