- CHAPTER VIII -

PHARMACODYNAMIC EFFECTS AND PHARMACOKINETIC PROFILE OF A LONG-TERM CONTINUOUS RATE INFUSION OF RACEMIC KETAMINE IN HEALTHY CONSCIOUS HORSES

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SUMMARY

Ketamine possesses analgesic and anti-inflammatory activity at sub-anaesthetic doses, suggesting a benefit of long-term ketamine treatment in horses suffering from pain, inflammatory tissue injury and/or endotoxaemia. However, data describing the pharmacodynamic effects and safety of continuous rate infusion (CRI) of ketamine and its pharmacokinetic profile in non-premedicated horses are missing. Therefore, we administered to six healthy horses a CRI of 1.5 mg/kg/h ketamine over 320 min following initial drug loading. Cardiopulmonary parameters, arterial blood gases, glucose, lactate, cortisol, insulin, non-esterified fatty acids, and muscle enzyme levels were measured, as were plasma concentrations of ketamine and its metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Levels of sedation and muscle tension were scored. Respiration and heart rate significantly increased during the early infusion phase. Glucose and cortisol significantly varied both during and after infusion. During CRI, all horses scored 0 on sedation. All but one horse scored 0 on muscle tension, with one mare scoring 1. All other parameters remained within or close to physiological limits without significant changes from pre-CRI values. Mean plasma concentration of ketamine during the 1.5 mg/kg/h ketamine CRI was 235 ng/mL. Decline of its plasma concentration-time curve of both ketamine and norketamine following the CRI was described by a two-compartmental model. The metabolic cascade of ketamine was norketamine, hydroxynorketamine and 5,6-dehydronorketamine. The ketamine median elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were 2.3 and 67.4 min, respectively. The area under the ketamine plasma concentration-time curve (AUC) was 76.0 μg•min/mL. Volumes of C_1 and C_2 were 0.24 and 0.79 L/kg, respectively. It was concluded that a ketamine CRI of 1.5 mg/kg/h can safely be administered to healthy conscious horses for at least 6 h, although a slight modification of the initial infusion rate regimen may be indicated. Furthermore, in the horse, ketamine undergoes very rapid biotransformation to norketamine and hydroxynorketamine and 5,6-dehydronorketamine were the major terminal metabolites.

1. INTRODUCTION

Ketamine [(±) 2-(ortho-chlorophenyl)-2-(methylamino)-cyclohexanone], a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has been in use in equine practice for both the induction and maintenance of general anaesthesia in horses for many years.[1,2] It causes a characteristic anaesthetic state often referred to as dissociative anaesthesia. Based on the observation that NMDA receptors are involved in the modulation of intractable visceral and somatic pain,[3-5] ketamine as one of the few clinically available NMDA receptor antagonists drugs has been tested and found to be effective as an analgesic in both laboratory and human clinical studies.[6-16]

Experimental evidence is surfacing that indicates that ketamine also exhibits potent anti-inflammatory actions including reduced chemotactic activation of neutrophils and suppression of leukocyte-dependent production of cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-8 through either direct or indirect mechanisms.[17-22] In an equine macrophage cell line, ketamine inhibited LPS-induced TNF-α and IL-6 responses in a concentration-dependent manner,[23] demonstrating that this drug could have cytokine-modulating effects in horses suffering from systemic inflammation and endotoxaemia. Considering the concentration-dependent influence and the fact that elevated plasma cytokine levels have been detected in horses for several hours after onset of endotoxaemia,[24,25] one must assume that ketamine has to be administered over extended periods of time and at relatively high doses to be clinically effective.

The analgesic and anti-inflammatory effects of ketamine, shown at sub-anaesthetic doses in other species, may prove useful in pain treatment and/or intra- and postoperative intensive care of the horse. Despite those attractive properties, the risk of side effects, mainly affecting the central nervous system (excitement/sedation) and increased skeletal muscle tone (catatonia), may preclude a wider use of ketamine for long-term treatment in the conscious horse.

We undertook the present study in preparation for future investigations analysing the immunomodulatory role of ketamine and its metabolites in vivo and focused on three main goals. First, we aimed to determine the safety of a continuous rate infusion (CRI) of racemic ketamine in conscious horses by evaluating its effects on central and peripheral nervous activity, cardiopulmonary function, metabolic and endocrinological homeostasis, choosing an infusion dose likely to achieve plasma concentrations that are known to exhibit analgesic and cytokine-modulating effects. Secondly, we aimed to expand the current knowledge regarding the metabolism of ketamine by using a modified version of a recently developed liquid

chromatography-mass spectrometry (LC-MS/MS) technique[26] to identify all metabolites that are formed in the horse. Thirdly, we aimed to determine the pharmacokinetic parameters for ketamine following steady-state infusion as those data are not yet reported for the conscious horse and are important for future dose tailoring.

2. MATERIALS AND METHODS

2.1. ANIMALS

Six healthy, adult Dutch Warmblood horses (four mares, two geldings, age 13 ± 6 years, body weight 600 ± 35 kg) in good body condition were studied after approval by the Utrecht University Ethics and Animal Research Committee. No food or water was withheld before or during the experiment.

2.2. ANIMAL PREPARATION AND INSTRUMENTATION

Two hours prior to experimentation, horses were restrained in stocks and a 12-G, 8 cm polytetrafluoroethylene catheter (Intraflon 2®, Vygon Nederland BV, Veenendaal, The Netherlands) was placed aseptically into the left and right jugular veins. Using lidocaine-prilocaine cream (EMLA®, Astra Pharmaceutica BV, Zoetermeer, The Netherlands) and an aseptic technique, a 14-G, 4.5-cm over the needle catheter (Arterial Cannula with FloSwitch®, Becton & Dickenson, Swindon, UK) was inserted percutaneously into the left facial artery for arterial blood sampling and arterial blood pressure (ABP) measurements. Respiration rate (RR) was determined on visual observation. The heart rate (HR) was recorded using a digital heart rate monitor (Polar HorseTrainer S610™, Polar Electro Europe BV, Fleurier, Switzerland). Systolic, diastolic and mean ABPs (SAP, DAP, MAP) were monitored using a disposable blood pressure transducer (DTXTM Plus DT-XX®, Becton & Dickinson Critical Care Systems Pte Ltd., Singapore, China) connected to a multichannel recorder (Hewlett-Packard®, Utrecht, The Netherlands). After restricting the horse's head height to its normal, relaxed standing position, the zero level of the transducer was set at the estimated level of the heart base. Transducers were calibrated before each experimental period for each horse.

2.3. EXPERIMENTAL PROTOCOL AND MEASURED PARAMETERS

All studies were started at 10:00 a.m. to avoid the possible variation imposed by circadian rhythm changes which have been identified in horses placed in a stable environment and accustomed to routine patterns of management.[27] Following

complete instrumentation, horses were allowed to adapt to the new environment for 60 min. A jugular venous infusion of racemic ketamine (Narketan® 100 mg/mL, Vétoquinol BV, 's-Hertogenbosch, The Netherlands), dissolved in 3 L of 0.9 % NaCl solution, was started in all animals at 10:00 a.m. (t = 0 min) at a rate of 4.8 mg/kg/h using a computer-controlled infusion device (ARGUS414®, Adquipment Medical B.V., Spijkenisse, The Netherlands). The infusion rate was then reduced in a step-wise fashion every 10 min to 3.6, 3.0, 2.4, and finally 1.5 mg/kg/h was maintained for 320 min (Fig. 1).

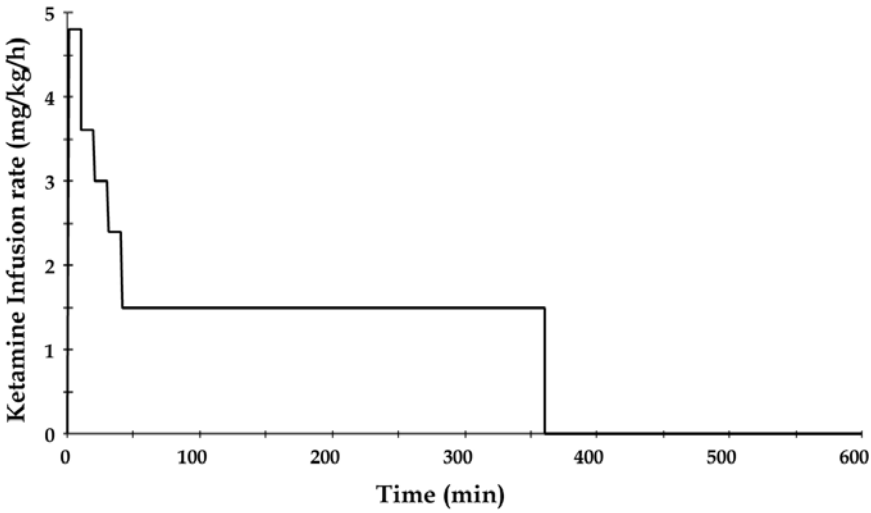

Figure 1. An intravenous infusion (CRI) of ketamine was started in each horse at 10:00 a.m. (time 0) at a loading rate of 4.8 mg/kg/h and then reduced in a step-wise fashion every 10 min till finally a rate of 1.5 mg/kg/h was achieved, which then was kept constant for 320 min.

All parameters including RR, HR, SAP, DAP, MAP, with the exception of muscle enzymes, were determined 15 min before starting ketamine infusion to collect baseline values. Baseline plasma concentrations of muscle enzymes were determined 24 h before start of infusion. Subsequent measurements of physiological and blood parameters were conducted at variable intervals. The level of sedation and muscle tension were subjectively scored each time RR and HR were recorded. A five-point scale (0-4) was used to score the level of sedation: 0 = no sedative effect, 1 = reduced alertness with no other signs, 2 = drowsiness and slight drop of the head, 3 = marked drowsiness and drop of the head, 4 = recumbency.[28] A four-point scale (0-3) was used

to score muscle tension: 0 = muscle relaxation present in trunk and limbs, 1 = muscle twitching present in some regions of trunk and limbs, 2 = muscle twitching present over the majority of trunk and limbs, 3 = muscle rigidity present over the majority of trunk and limbs.[29]

Blood samples for arterial pH (pH), partial pressures of O_2 (P_aO_2) and CO_2 (P_aCO_2) and venous glucose and lactate levels were collected in heparinised syringes (2 mL) and immediately analysed using a blood gas analyzer (ABL System 605®, Radiometer, Copenhagen, Denmark). Heparinised venous blood samples were collected for measurement of plasma lactate dehydrogenase (LDH), creatine phosphokinase (CK) and aspartate aminotransferase (AST) levels. Blood samples were centrifuged immediately following collection and then, plasma was harvested and stored at -20 °C until analysis. Plasma concentrations of LDH, CK, and AST were determined later by a colorimetric technique. Venous heparinised blood samples (8 mL) for cortisol, insulin and non-esterified fatty acids (NEFA) determinations were centrifuged immediately following collection, plasma was harvested and then stored at -20 °C until analysis. Plasma insulin and cortisol concentrations were measured by use of commercial radioimmunoassay kits (Coat-A-Count® Insulin and Coat-A-Count® Cortisol; Diagnostic Products Corporation, Los Angeles, USA), and NEFA concentrations were measured by colorimetry using a Randox kit (Randox Laboratories Ltd, Ardmore, UK). All three kits were validated for samples obtained from horses.[30]

Two blood samples for measurement of ketamine and its metabolites in plasma were collected prior to drug administration and at 9, 19, 29, 39, 45, 50, 90, 150, 210, 270, 330, 359, 361, 363, 365, 368, 372, 380, 395, 410, 430, 450, 480, 510, 540, 570, and 600 min after start of ketamine infusion. Samples were centrifuged, serum harvested and then stored at -70 °C prior to LC-MS/MS analysis.

2.4. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS) ANALYSIS

Dichloromethane (Cert ACS/HPLC grade), isopropanol (ACS/HPLC grade), ammonium hydroxide (ACS grade), 16 x 125 screw cap culture tubes, and 16 x 100 screw cap tubes were obtained from Scientific Equipment Company (SECO, Aston, PA, USA). Formic acid (Supra-Pure, 99%) was obtained from VWR Scientific (Bridgeport, NJ, USA). Water and acetonitrile (Optima grade) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Calibration standards for ketamine HCl, norketamine HCl, and d_4-ketamine (internal standard) were obtained from Cerriliant Corp. (Austin, TX, USA). All reagents, solutions, and standards were prepared from these materials.

Unknowns, calibrators, and controls were prepared by adjusting 0.1 mL samples to pH of 9.0 with 2 mL of pH 9.0 ammonium formate placed in screw cap tubes. Internal standard d4-ketamine (100 µL) was added to all tubes (unknowns, calibrators and controls) except negative control. Calibrators and controls were prepared by using 0.1 mL aliquots of previously prepared spiked plasma (1-1000 ng/mL). All tubes were then extracted with 5 mL of dichloromethane:isopropanol (10:1) on gentle mixing (rotorack) for 10 min. The sample tubes were centrifuged for 5 min at 2500 x g, after which the organic solvent was transferred to clean, dry, labeled culture tubes and evaporated at 50 ^{o}C under a gentle stream of nitrogen. The dried samples were reconstituted with 0.1 mL 0.1 % formic acid, and then transferred to clean, labeled auto sampler vials, fitted with 0.2 mL limited volume inserts and capped.

Previously screened negative equine plasma sample was used for the creation of calibrators and positive controls. Ten concentrations, ranging from 1 to 1000 ng/mL, were prepared. Aliquots of 0.1 mL of each sample were used to maintain the analysis within the linear range of the instrumentation with sensitivity sufficient for the current studies.

Analysis was conducted on an integrated liquid chromatograph-mass spectrometer. The liquid chromatograph with auto sampler was a Surveyor® (Thermo Electron Corp., San Jose, CA, USA) and the mass spectrometer was a Deca XP plus® (Thermo Electron Corp., San Jose, CA, USA) operated in positive ion electrospray mode. The chromatographic column was an ACE 5 C-18 analytical column (3 x 50 mm, 5 micron) and the integrated guard column an ACE 3 C-18 (2.1 x 12.5 mm). The LC mobile phase was 2.33 mM formic acid (pH 6.0) and acetonitrile, run over a 10-min convex gradient from 100 % aqueous to 100 % organic at 0.2 mL/min. The mass spectrometer utilised atmospheric pressure ionisation (API) in positive ion electrospray mode, and data were acquired by reconstructed ion chromatograms of targeted precursor ions in MS mode. Data reconstruction and quantification was performed using Xcalibur® software, version 1.3 (Thermo Electron Corp., San Jose, CA, USA).

Chromatographic conditions were determined in MS mode by extraction of the relevant precursor ion species [M + H^+] for the respective analytes. Peak purity was determined by examination of the relative isotopic abundance of the precursor ion and the chlorine isotope [M + H^+ + 2] ion. This was necessary to determine full chromatographic separation of isobaric analytes with either [M + H^+] or [M + H^+ + 2] masses at the masses of m/z 224, 226, 240, and 242.

The MS mode allowed the determination of analytes for which no reference compound exists for the construction of calibration curves. For calibration of hydroxynorketamine and 5,6-dehydronorketamine the norketamine calibration curve was utilised. The ionisation efficiencies of MS mode precursor ion production are more parallel than the tandem MS/MS mode, which would be dependent upon both the efficiencies of product ion generation, as well as the product ions chosen for quantitative determination. Standard operating procedures for the quantification of analytes met requirements for accreditation by the American Association for Laboratory Accreditation and International Guidelines (ISO/IEC 17025, Geneva, Switzerland, 1999).

2.5. PHARMACOKINETIC ANALYSIS

Plasma concentration-time curve of ketamine and its metabolites, norketamine, hydroxynorketamine, and 5,6-dehydronorketamine following IV infusion of ketamine were analysed using standard linear compartmental analysis (WinSAAM).[31,32] Pharmacokinetics of ketamine and its metabolites were described by a six-compartment model (Fig. 2). The infusion of ketamine was into compartment 1 (C_1) and the distribution of ketamine and norketamine were described by two-compartment inter-compartmental transfer rate constants $k_{1,2}$, $k_{2,1}$, $k_{3,4}$ and $k_{4,3}$.

The rate of metabolism and appearance of norketamine from ketamine was estimated by $k_{1,3}$ and the rate of direct elimination and metabolism of norketamine to hydroxynorketamine and 5,6-dehydronorketamine were described by $k_{3,0}$, $k_{3,5}$, and $k_{3,6}$, the sum of which in effect describes the total elimination of norketamine. The elimination of hydroxynorketamine and 5,6-dehydronorketamine from C_5 and C_6 was described by fractional rate constants $k_{5,0}$ and $k_{6,0}$.

The inter-compartmental fractional transfer rates were directly estimated by using the following equations:

$$\dot{Y} = -LY + U \qquad (1)$$

where Y is a matrix describing the quantity of drug present in each of the compartments considered, $\dot{Y}$ is the derivative of Y, L is a matrix of the fractional rate constants describing the inter-compartmental flow of drug, and U is a matrix of the instantaneous input rates into each compartment. L is related to the eigenvalues and eigenvectors by:

$$L = A \alpha A^{-1} \qquad (2)$$

where A is the matrix of eigenvectors (boundary conditions), α is a matrix of the eigenvalues (exponential slope constants) and A^{-1} is the inverse of A.[33] The WinSAAM software is uniquely refined to permit direct translation between the exponential and equivalent compartmental model forms. The eigenvalues were estimated using WinSAAM utilising equations 1 and 2. Half-lives ($t_{1/2}$) were calculated as the natural $\log_2$ divided by the fractional rate constants.

The weights W_k, applied in the fitting process utilised the fractional standard deviation (FSD) of the data, and were in the form of $W_k = 1/[C \times Q_{O(k)} \times 2]$, in which $Q_{O(k)}$ is the kth observed datum and C is its FSD. The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration is < 1%.[33]

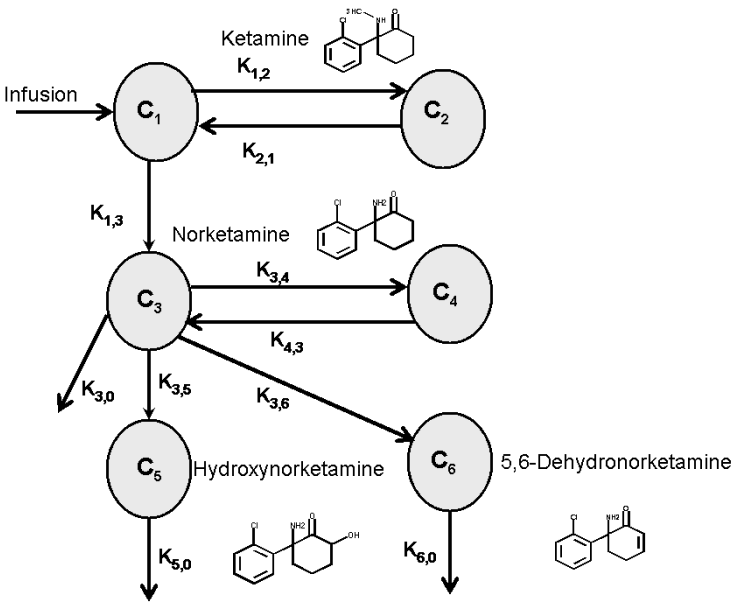

Figure 2. The six-compartment model describing the distribution of ketamine and its metabolites following the intravenous infusion of ketamine. The infusion of ketamine was into compartment 1 (C_1) and the distribution and elimination of ketamine and norketamine were described by two-compartment inter-compartmental transfer rate constants $k_{1,2}$, $k_{2,1}$, $k_{3,4}$ and $k_{4,3}$. The rate of metabolism and appearance of norketamine from ketamine was estimated by $k_{1,3}$. The rate of direct renal elimination of norketamine and metabolism of norketamine to hydroxynorketamine and 5,6-dehydronorketamine were described by $k_{3,0}$, $k_{3,5}$, and $k_{3,6}$, the sum of which in effect describes the total elimination of norketamine. The elimination of hydroxynorketamine and 5,6-dehydronorketamine from C_5 and C_6 were described by fractional rate constants $k_{5,0}$ and $k_{6,0}$. Volumes (V) of C_3, C_5, and C_6 were set equal to C_1.

The total area under the plasma ketamine and metabolites concentration curves (AUC_0^{610}) from 0 to 610 min was calculated using the trapezoid rule. Time to maximum plasma concentration (T_{max}) and maximum plasma concentration (C_{max}) were obtained directly from the experimental data. Volumes (V) of C_3, C_5, and C_6 were set equal to C_1. The volumes of C_2 and C_3 were calculated as:

$$V_2 = V_1 \times k_{1,2}/k_{2,1} \text{ and } V_3 = V_1 \times k_{3,4}/k_{4,3}$$

Clearance (Cl) from each compartment was estimated as:

$$Cl = V \times k$$

2.6. STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS© 12.01 statistical package (SPSS© Inc., Chicago, IL, USA). Pharmacodynamic data and the plasma concentrations of ketamine and its metabolites, norketamine, hydroxynorketamine, and 5,6-dehydronorketamine, were analysed using a repeated measures analysis of variance and the Huynh-Feldt test statistic to adjust for sphericity and were expressed as mean ± standard deviation (S.D.). When significant a paired t-test was used to compare differences with baseline values. Pharmacokinetic parameter estimates of ketamine and metabolites were expressed as median and range. Differences were considered to be significant when $p<0.05$.

3. RESULTS

3.1. CARDIOVASCULAR, METABOLIC AND ENDOCRINE EFFECTS

In all horses RR and HR significantly increased during the early infusion phase but after 20 min, when the ketamine infusion rate was decreased to 3.6 mg/kg/h, values returned to baseline and remained stable for the remainder of the experiment, reaching a significant minimum for RR at 30 h (Table 1). SAP, DAP, MAP and arterial blood gas tensions were collected in five horses only as in one horse arterial catheterisation was not successful. Despite initial mild blood pressure increases and subsequent increases towards the end of ketamine infusion period, none of the time points were statistically significant from control (Table 1). The pH, P_aO_2 and P_aCO_2 were within physiological ranges throughout most of the infusion period, only P_aCO_2 showed significant difference from baseline at 360 min of ketamine infusion.

Plasma lactate, insulin and NEFA concentrations remained within physiological limits, with the only exception of a significant decrease of plasma NEFA concentrations at 30 h post-infusion (Table 2). Throughout the entire experiment, plasma glucose concentrations remained slightly above the physiological range (3.9-5.6 mmol/L). Plasma cortisol concentrations varied throughout the experimental period.

Table 1. Cardiopulmonary and blood-gas parameters prior to, during, and following ketamine continuous rate infusion in conscious horses

Time	RR† (min^{-1})	HR† (min^{-1})	SAP† (mmHg)	DAP (mmHg)	MAP† (mmHg)	pH	P_aO_2 (kPa)	P_aCO_2† (kPa)
Baseline	16 ± 5	37 ± 3	144 ± 21	89 ± 9	112 ± 16	7.42 ± 0.01	13.6 ± 1.0	5.3 ± 0.5
10 min	21 ± 5*	51 ± 9*	165 ± 14	97 ± 18	119 ± 11			
20 min	22 ± 4	53 ± 13*	165 ± 20	95 ± 6	126 ± 13			
30 min	22 ± 3	51 ± 18	165 ± 26	89 ± 5	126 ± 16			
60 min	20 ± 5	44 ± 9	151 ± 16	87 ± 12	115 ± 11	7.43 ± 0.04	13.7 ± 1.9	5.2 ± 0.4
90 min	17 ± 6	41 ± 8	148 ± 24	83 ± 10	109 ± 10			
120 min	17 ± 5	40 ± 4	144 ± 14	87 ± 14	108 ± 10	7.43 ± 0.01	13.1 ± 1.0	5.2 ± 0.3
150 min	17 ± 4	37 ± 4	135 ± 7	86 ± 9	108 ± 11			
180 min	16 ± 4	38 ± 4	143 ± 10	87 ± 12	108 ± 10	7.43 ± 0.02	13.9 ± 2.3	5.2 ± 0.6
210 min	17 ± 4	39 ± 7	132 ± 10	82 ± 9	105 ± 6			
240 min	15 ± 5	37 ± 3	139 ± 7	85 ± 16	106 ± 9	7.44 ± 0.02	13.2 ± 0.7	5.2 ± 0.4
270 min	16 ± 4	39 ± 4	143 ± 10	89 ± 6	110 ± 6			
300 min	19 ± 6	40 ± 5	149 ± 16	90 ± 15	112 ± 9	7.42 ± 0.01	12.8 ± 0.8	5.5 ± 0.4
330 min	17 ± 4	41 ± 5	150 ± 17	94 ± 12	117 ± 10			
360 min	17 ± 2	41 ± 5	157 ± 15	94 ± 12	120 ± 12	7.44 ± 0.04	14.9 ± 1.7	4.8 ± 0.3*
370 min	17 ± 5	43 ± 4	148 ± 16	89 ± 9	113 ± 11			
380 min	17 ± 2	40 ± 4			113 ± 22			
390 min	17 ± 4	40 ± 6			115 ± 10			
14 h	14 ± 3	35 ± 3						
30 h	14 ± 5*	34 ± 2						

Values are expressed as mean ± S.D..
RR = respiration rate; HR = heart rate; SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = mean arterial pressure; PaO_2 = arterial partial pressure of oxygen; P_aCO_2 = arterial partial pressure of carbon dioxide.
† Parameters changing significantly from baseline over time based on repeated measures analysis of variance and Huynh-Feldt tests.
* Values significantly different (based on paired t-test) from baseline recorded 15 min prior to start of ketamine infusion: $p<0.05$.

Table 2. Lactate, glucose, insulin, cortisol, non-esterified fatty acid (NEFA), and muscle enzyme levels prior to, during, and following ketamine continuous rate infusion in conscious horses

Time (h)	Lactate (mmol/L)	Glucose† (mmol/L)	Insulin (pmol/L)	Cortisol† (nmol/L)	NEFA† (mmol/L)	LDH (IU/L)	AST (IU/L)	CK (IU/L)
Baseline	0.7 ± 0.3	7.2 ± 0.8	393 ± 339	287 ± 91	0.23 ± 0.16	538 ± 151	165 ± 50	152 ± 63
1	0.7 ± 0.3	5.9 ± 1.1*	218 ± 172	201 ± 56*	0.38 ± 0.09			
2	0.8 ± 0.3	6.0 ± 0.5*	250 ± 169	178 ± 46*	0.28 ± 0.16			
3	0.7 ± 0.3	5.9 ± 0.3*	235 ± 160	144 ± 34*	0.20 ± 0.07	683 ± 465	179 ± 50	175 ± 94
4	0.6 ± 0.2	5.8 ± 0.2*	187 ± 151	152 ± 60*	0.20 ± 0.08			
5	0.5 ± 0.1	5.7 ± 0.5*	155 ± 110	168 ± 76*	0.22 ± 0.07			
6	0.6 ± 0.2	5.8 ± 0.4*	127 ± 88	214 ± 40	0.27 ± 0.11	438 ± 130	163 ± 63	144 ± 62
7	0.6 ± 0.2	5.8 ± 0.6*	104 ± 85	271 ± 49	0.27 ± 0.12			
8	0.7 ± 0.1	6.0 ± 0.9*	148 ± 125	201 ± 55*	0.25 ± 0.17			
10	0.8 ± 0.1	6.0 ± 0.3*	151 ± 75	133 ± 48*	0.11 ± 0.05	567 ± 137	165 ± 33	158 ± 43
14	0.7 ± 0.1	5.6 ± 0.4*	134 ± 56	121 ± 32*	0.08 ± 0.01			
22	0.7 ± 0.1	5.8 ± 0.7*	192 ± 100	204 ± 33	0.10 ± 0.02			
30	0.8 ± 0.2	5.8 ± 0.3*	183 ± 74	135 ± 50*	0.07 ± 0.03*	440 ± 185	189 ± 41	130 ± 40

Values are expressed as mean ± S.D..
NEFA = non-esterified fatty acids; LDH = lactate dehydrogenase; CK = creatine kinase; AST = aspartate aminotransferase.
† Parameters changing significantly from baseline over time based on repeated measures analysis of variance and Huynh-Feldt tests.
* Values significantly different (based on paired t-test) from baseline recorded 15 min prior to start of ketamine infusion: $p<0.05$.

3.2. BEHAVIORAL EFFECTS

All horses started shifting weight from one front leg to the other within 5-10 min after start of infusion and continued to show this behavior for 20-30 min. During ketamine CRI, none of the horses displayed any sedative effects. On the contrary, upon subjective evaluation, three of six horses showed an increase in alertness during the 6-h infusion period.

3.3. SKELETAL MUSCLE

All but one horse scored 0 on muscle tension. One mare scored 1 at 20 and 30 min following start of ketamine infusion. There were no significant changes in plasma LDH, CK and AST concentrations 3 h after the start of infusion (Table 2).

3.4. KETAMINE PHARMACOKINETICS AND METABOLISM

The median and range of the inter-compartmental fractional rate constants and pharmacokinetic parameter estimates for the IV infusion of ketamine and its metabolites are shown in Tables 3 and 4. The plasma concentration of ketamine progressively decreased as the rate of infusion was decreased and this change was significant ($p<0.02$) at the end of the 2.4 mg/kg/h infusion period (Table 5; Fig. 3). Conversion of ketamine to norketamine was quantified at the first sampling time and there was a significant increase in the norketamine plasma concentrations ($p<0.02$) at the end of first infusion period of ketamine (4.8 mg/kg/h). The norketamine concentrations gradually declined and paralleled the concentration changes of ketamine (Fig. 4). Conversion of norketamine to hydroxynorketamine and 5,6-dehydronorketamine was also rapid and continuous over the CRI (Table 5; Figs 3 and 4). There were significant increases in the plasma concentration of hydroxynorketamine ($p<0.04$) and 5,6-dehydronorketamine ($p<0.01$) at the end of the second infusion period of ketamine (3.6 mg/kg/h) (Table 5). There were no significant differences in the plasma concentrations of hydroxynorketamine and 5,6-dehydronorketamine at the end of the CRI.

Once the CRI was discontinued, ketamine concentrations decreased rapidly, reaching plasma levels approximately one-tenth of those measured during CRI within 90 min. A six-compartment model best described the decline of the plasma concentration-time curve of ketamine and its metabolites (Fig. 2). A biexponential equation best described the data for ketamine and norketamine. The initial distributive (α) phase of ketamine was characterised by an estimated half-life of 2.3 min ($t_{1/2\alpha}$) and the slower elimination (β) phase by a half-life of 67.4 min ($t_{1/2\beta}$) (Table 3). Thus, ketamine distributed very rapidly from the central (C_1; plasma and blood-rich tissues) to the peripheral tissue compartments (C_2) with an inter-compartmental transfer rate constant $k_{1,2}$ of 0.046 min^{-1}. The apparent volumes of C_1, C_2 and C_4 were estimated as 242.2, 786.2, and 755.1 mL/kg, respectively, and the estimated clearances of C_1, C_3, C_5, and C_6 are also shown (Table 3). Like the parent drug, norketamine rapidly interchanged between the central and peripheral compartments (C_3 and C_4). The elimination of the secondary metabolites hydroxynorketamine and 5,6-dehydronorketamine were described by a mono-exponential equation (Fig. 3; Table 4).

Table 3. Pharmacokinetic parameter estimates of ketamine and metabolites following an IV infusion of ketamine in n = 6 horses

Parameter	Median	Range
Ketamine		
$t_{1/2\alpha}$ (min)	2.34	1.67-2.86
$t_{1/2\beta}$ (min)	67.45	59.2-85.6
$t_{1/2kt\,1}$ (min)	2.96	2.29-3.31
V_1 (mL/kg)	242.2	163.7-335.8
V_2 (mL/kg)	786.2	329.4-1437.7
Cl_1 (mL/min/kg)	53.0	36.6-88.7
AUC_0^{610} (μg•min/mL)	76.0	48.0-105.3
Norketamine		
$t_{1/2\alpha}$ (min)	10.46	5.0-15.3
$t_{1/2\beta}$ (min)	160.69	80.2-307.6
$t_{1/2kt\,3}$ (min)	7.53	5.4-9.0
V_4 (mL/kg)	755.1	547.3-1558.2
Cl_3 (mL/min/kg)	23.3	15.9-42.9
AUC_0^{610} (μg•min/mL)	160.8	94.1-232.9
Hydroxynorketamine		
$t_{1/2ke5}$ (min)	56.95	33.6-64.4
Cl_5 (mL/min/kg)	3.1	2.0-6.8
AUC_0^{610} (μg•min/mL)	372.9	304.5–449.3
5,6-Dehydronorketamine		
$t_{1/2ke6}$ (min)	43.26	27.6-46.5
Cl_6 (mL/min/kg)	4.4	2.6-8.1
AUC_0^{610} (μg•min/mL)	396.6	251.1-568.2

$t_{1/2\alpha}$ and $t_{1/2\beta}$ = compartmental half-lives; $t_{1/2kt}$ = transfer half-lives from C_1 and C_3; $t_{1/2ke}$ = elimination half-lives from C_5 and C_6; V_1 and V_2= apparent volumes of C_1 and C_2; AUC = area under the curve; Cl = clearance from V_1, V_3, V_5, and V_6.

Table 4. Median and range of calculated intercompartmental transfer and elimination rate constants (k) and fractional standard deviation of the estimates (FSD) of the six-compartment model describing the pharmacokinetics of ketamine and metabolites following the IV infusion of ketamine in n = 6 horses

Parameter	Median	Range	FSD
Ketamine			
$k_{1,2}$ (min^{-1})	0.046	0.015-0.060	0.034
$k_{2,1}$ (min^{-1})	0.013	0.009-0.015	0.015
$k_{1,3}$ (min^{-1})	0.235	0.209-0.303	0.017
Norketamine			
$k_{3,4}$ (min^{-1})	0.033	0.015-0.140	0.057
$k_{4,3}$ (min^{-1})	0.010	0.006-0.019	0.058
$k_{3,5}$ (min^{-1})	0.032	0.021-0.062	0.019
$k_{3,6}$ (min^{-1})	0.044	0.036-0.066	0.018
$k_{3,0}$ (min^{-1})*	0.013	0.000-0.038	0.068
Hydroxynorketamine			
$k_{5,0}$ (min^{-1})	0.012	0.011-0.021	0.016
5,6-Dehydronorketamine			
$k_{6,0}$ (min^{-1})	0.016	0.015-0.025	0.016

Table 5. Plasma concentrations (mean ± S.D.) of ketamine (KET) and its metabolites norketamine (NKET), hydroxynorketamine (HNK), and 5,6-dehydronorketamine (DHNK) at the end of each infusion period (n = 6 horses)

Infusion Rate (mg/kg/h)	Time (min)	KET (ng/mL)	NKET (ng/mL)	HNK (ng/mL)	DHNK (ng/mL)
4.8	9	407 ± 183	351±154*	184 ± 57	159 ± 62
3.6	19	368 ± 87	471 ± 137	315 ± 63*	347 ± 74*
3.0	29	352 ± 88	538 ± 115	460 ± 91	535 ± 99
2.4	39	288 ± 80*	510 ± 120	506 ± 83	649 ± 144
1.5	372	230 ± 120	489 ± 137	920 ± 169	1001 ± 238

*Values significantly different from measurements at previous infusion rate and sampling time, respectively: $P < 0.05$.

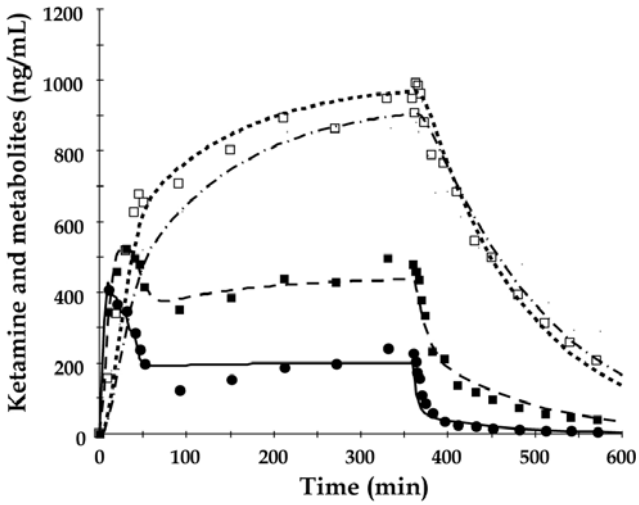

Figure 3. Plasma concentrations and the corresponding curves of best fit of ketamine (●—), norketamine (■---), hydroxynorketamine (○— ▪ — ▪ —), and 5,6-dehydronorketamine (□▪▪▪▪▪) during and following IV ketamine infusion. Symbols represent mean values from n = 6 horses.

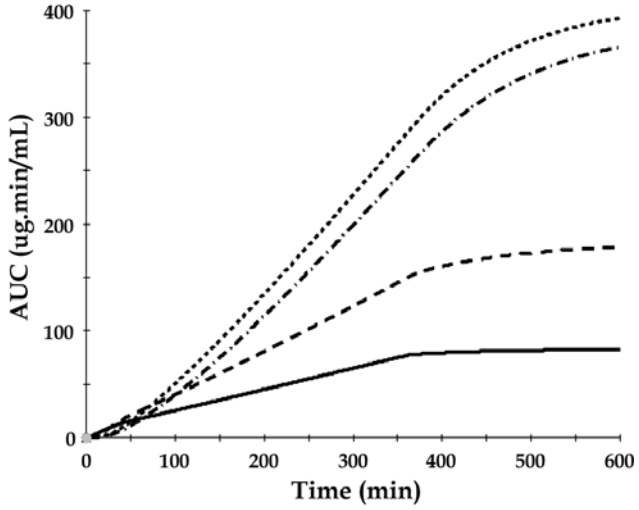

Figure 4. Area under the curve (AUC) values of ketamine and its metabolites during and following IV ketamine infusion. The AUC reflects the relative amount of metabolites to the parent compound (bottom to top: ketamine, norketamine, hydroxynorketamine, and 5,6-dehydronorketamine). Data from n = 6 horses.

4. DISCUSSION

4.1. PHARMACODYNAMIC EFFECTS OF RACEMIC KETAMINE DURING LONG-TERM INFUSION IN CONSCIOUS HORSES

The present data indicate that in healthy conscious horses ketamine at a rate of 1.5 mg/kg/h can be safely administered over prolonged periods of time. The CRI rate of 1.5 mg/kg/h chosen in this study was based on previously published data, in which horses were receiving an IV induction dose of 2.2 mg/kg and returned to consciousness when plasma ketamine concentrations fell to or below 1 μg/mL.[34] Thus, to be prepared for future investigations analysing dose-dependent immunomodulatory effects of ketamine in the horse, we aimed at achieving relatively high plasma concentrations, yet without the risk of causing loss of consciousness. A 0.8 μg/mL plasma concentration appeared to be the upper limit, as it was close to but still safely below those associated with anaesthetic effects. Applying the previously reported pharmacokinetic variables in the horse[34] (Table 6), a computer program for therapeutic drug monitoring (Beal SL, Sheiner LB: NONMEM Users Guides, NONMEM Project Group, University of California, San Francisco, CA, USA) predicted a ketamine CRI dose of 1.5 mg/kg/h as appropriate for achieving this goal. To reach steady-state concentrations, a CRI can either be preceded by a bolus injection or by an initially higher infusion rate, followed by a stepwise decrease of the dosage. We opted for the latter, as it carried the least risk of resulting in peak plasma concentrations in excess of 1 μg/mL.

During the 1.5 mg/kg/h ketamine CRI plasma ketamine concentrations averaged 235 ng/mL (range: 118-277 ng/mL; Figs 3 and 4), which were far below the plasma concentrations predicted by the computer program. This significant discrepancy might have been caused by a variety of reasons. Foremost, pharmacokinetic properties may change when ketamine is administered in combination with other drugs, leading to altered metabolism, distribution and excretion of the compound.

Table 6. Pharmacokinetic data for racemic ketamine from comparative references

	Kaka et al., 1979[35] (n = 4)	Waterman et al., 1987[34] (n = 10)	Schwieger et al., 1991[41] (n = 4)		Domino et al., 1982[†,46] (n = 7)
Species	Horse	Horse	Dog		Human
Ketamine dose	2.2 mg/kg IV	2.2 mg/kg IV	10 mg/kg IV	0.3 mg/kg/min CRI[*]	2.0-2.2 mg/kg IV
V_1 (mL/kg)	212 ± 24	492 ± 74	810 ± 135	n.d.	50 ± 17
V_2 (mL/kg)	611 ± 131	n.d.	n.d.	2888 ± 460	157 ± 47
V_{SS} (mL/kg)	1625 ± 103	611 ± 131	1370 ± 205	n.d.	2114
Cl (mL/min/kg)	26.6 ± 3.5	31.1 ± 2.3	18.1 ± 2.9	13.9 ± 2.5	18.6
$t_{1/2\alpha}$ (min)	2.9	2.89 ± 0.25	7.7 ± 0.9	n.d.	0.49 ± 0.08
$t_{1/2\beta}$ (min)	42	65.84 ± 3.46	121.9 ± 4.6	140.6 ± 19.8	158 ± 36
$k_{1,2}$ (min^{-1})	0.093 ± 0.020	0.164 ± 0.024	n.d.	n.d.	n.d.
$k_{2,1}$ (min^{-1})	0.032 ± 0.006	0.042 ± 0.005	n.d.	n.d.	n.d.

Data are presented as mean ± S.D..
V_1 and V_2 = the estimated volumes of distribution for the central and peripheral compartments, respectively; V_{SS} = the volume of distribution at steady-state; $t_{1/2\alpha}$ and $t_{1/2\beta}$ = the compartmental half-lives; $k_{1,2}$ and $k_{2,1}$ = the corresponding inter-compartmental transfer rate constants; Cl = an estimate of the total body clearance rate; n.d. = not determined.
[*] Initial ketamine loading dose of 26 mg/kg over 20 min, followed by a continuous rate infusion (CRI) for 5 h.
[†] Data were analysed using a three-compartment model.

Although plasma concentrations were far below the estimated concentration of 800 ng/mL, evaluation of the recorded physiological and behavioral parameters suggests that the CRI rate chosen approaches the upper limit of what may be tolerated under clinical circumstances. Especially the infusion rates of 4.8 mg/kg/h and 3.6 mg/kg/h, administered early during the infusion period, were associated with significant increases in RR, HR and ABP (Table 1). In addition, at these infusion rates, horses showed mild behavioral changes in the form of shifting weight from one front leg to the other, and some muscle twitching was recorded in both front legs of one horse. In addition, increased alertness observed in three of six horses during the 6-h ketamine infusion, points towards increased central nervous activity caused by ketamine and warrants caution when infusing the drug at higher rates.

High baseline plasma glucose and cortisol concentrations might indicate a stress response induced by experimental environment and manipulations performed prior to ketamine infusion. One may also speculate that the increase in plasma cortisol concentration, rather than the expected diurnal decrease, and the remaining elevated plasma glucose concentrations during the experiment were the result of the ketamine CRI, even though other factors not controlled for in these experiments might have been responsible as well. As an indirect sympathomimetic drug, ketamine may cause elevated endogenous catecholamine levels and hence stimulate the release of plasma cortisol, subsequently leading to hyperglycemia caused by increased gluconeogenesis and/or insulin resistance. In order to evaluate any acute impact of ketamine on skeletal muscle tissue integrity, plasma concentrations of LDH, AST and CK were measured. Creatine phosphokinase has a high specificity for muscle damage. Therefore, the facts that no enzyme showed a significant change of the plasma concentration, and that both AST and CK remained within physiological limits, suggests that ketamine, when administered at the infusion rates used in this study, had no significant impact on skeletal muscle activity and tissue integrity.

4.2. PHARMACOKINETICS

Clinically, ketamine is used primarily as a short-acting anaesthetic, most commonly administered in conjunction with α_2-adrenoceptor agonists. Thus, in previous equine studies, the drug was administered at anaesthetic doses (≥ 2.2 mg/kg) following an IV injection of either xylazine (1.1 mg/kg) or detomidine (20 μg/kg) and its pharmacokinetic profile was determined under conditions of inhalant or total intravenous anaesthesia.[34-36] Inhalant anaesthetics (e.g., halothane) and anaesthetic adjuvants (e.g., diazepam, secobarbital, alfentanil, xylazine) are

known to delay redistribution and hepatic metabolism of ketamine in various animal species. Chronic ketamine administration is associated with induction of hepatic drug-metabolising cytochrome P_{450} (CYP_{450}) enzymes in laboratory animals.[34,37,38] Moreover, protein binding of ketamine,[39] stability of ketamine in blood samples,[40] and accuracy of the analytical technique used to measure ketamine concentrations in plasma can be of influence. Thus, extrapolating previously obtained pharmacokinetic data for the use of long-term infusion of ketamine in otherwise non-premedicated horses may not be appropriate. The present study is therefore the first to investigate in awake horses the distribution and metabolism of ketamine when administered in the form of a long-term infusion (1.5 mg/kg/h).

Pharmacokinetic variables ($t_{1/2\alpha}$, $t_{1/2\beta}$, V_1, V_2, Cl) determined for infusion of ketamine were not very different from those previously reported in horses after IV bolus administration[34-36] (compare Tables 3 and 6). This observation coincides with findings in dogs,[41] in which clearance (Cl) and elimination half-lives ($t_{1/2\beta}$) were similar between animals receiving a single IV dose or a CRI of ketamine (Tables 3 and 6). Plasma concentrations only reached a stable plateau after 300 min of ketamine CRI (Fig. 3), confirming that for a true steady-state to occur continuous drug infusion must be maintained for a period of at least five elimination half-lives.[42-43] The average plasma ketamine concentration during 1.5 mg/kg/h ketamine infusion was 235 ng/mL and thus was far below levels needed to produce anaesthesia (i.e., 1.2 ± 0.2 μg/mL),[35] but was within the range of concentrations that exhibit analgesic effects in humans[15,16] and anti-inflammatory actions in vitro.[23] Similar to the results obtained in previous pharmacokinetic studies in horses[34-36] and other species,[37] following termination of the CRI plasma ketamine concentrations declined biexponentially, and the disposition of ketamine and norketamine in all six horses was best described using a two-compartment model, which had previously been described for ketamine in the horse,[34,35] dog,[44] cat,[45] and other species including man as well.[37] As with an IV bolus during CRI of ketamine the initial distribution phase from the central (plasma and vessel-rich tissues; C_1) to the peripheral tissue compartments (C_2) occurs with a very short half-life ($t_{1/2\alpha}$) of approximately 2-3 min, followed by a much slower elimination phase, representing both metabolism and renal elimination and occurring with a half-life ($t_{1/2\beta}$) of about 1 h. The apparent volume of distribution of the peripheral compartments (C_2 and C_4) was approximately 3.2 times greater than that of C_1 (Table 3), which has been also observed in other species such as the dog,[44] cat,[45]

and human.[46,47] The large volume of the second compartment is characteristic for the strong lipid solubility of weak bases such as ketamine[37] and hence confirms the high affinity of the drug for many tissues other than plasma.

Ketamine is metabolised extensively by the hepatic CYP_{450} enzyme system in all species including the horse.[37,48,49] However, total body clearance (Table 3) appears to exceed hepatic blood flow in the horse[35] and renal ketamine excretion occurs only in the immediate period post-IV administration,[50] indicating that also extrahepatic pathways in organs such as kidney, lung, and gut may participate in the elimination of ketamine from plasma, as has been demonstrated in the rat.[51] In many species including the horse,[37,49] initial hepatic biotransformation via N-demethylation produces norketamine (metabolite I) that then can be hydroxylated at one or more positions in the cyclohexanone ring to form hydroxynorketamine, which in turn can be conjugated to more water-soluble glucuronide derivatives and be excreted by the kidneys (Fig. 2). Since the hyroxylated metabolites of norketamine are unstable ex vivo at higher temperatures, they were thought to rapidly undergo further oxidation (thermal dehydration) to 5,6-dehydronorketamine, which had been identified as the predominant final metabolite also found in equine serum and urine following IV ketamine administration.[50,52]

Although called metabolite II, [34,37] the in vivo existence of 5,6-dehydronorketamine as a true metabolite has long been questioned by investigators suggesting that formation of 5,6-dehydronorketamine could be the result of an analytical artifact associated with the gas-liquid chromatography-mass spectrometry (GC-MS) that was commonly used at the time for detection and quantification of ketamine and metabolites in plasma and urine.[26,37] It was thought that under the harsh chemical conditions of the GC method, involving benzene extraction of a strongly alkalinised sample followed by heptafluorobutyric anhydride derivatisation in the presence of pyridine, hydroxynorketamine and other α- and β-hydroxy ketones would be easily converted to the respective dehydro compounds.[26] The use of the more benign LC-MS in this study has been shown to eliminate much of the thermal component of 5,6-dehydronorketamine generation. The difference between the two available methodologies can be quantified by comparison of 5,6-dehydronorketamine levels obtained by GC-MS and LC-MS/MS. Additionally, the lack of any GC-MS reports of hydroxynorketamine speaks directly to the facility with which this conversion can take place. The elevated temperatures of GC-MS instrumentation and the harsh conditions of chemical derivatisation both seem more than sufficient to produce 5,6-dehydronorketamine from hydroxy-

norketamine. The present study shows high levels of both hydroxynorketamine and 5,6-dehydronorketamine, which have parallel slopes of appearance and disappearance (Fig. 3). However, even using our LC-MS/MS method to minimise the thermal conversion, ion source conditions and electrospray ion formation and ejection processes may still be sufficient to facilitate the enol-mediated dehydration process, probably leading to some degree of artificial formation of 5,6-dehydronorketamine in addition to in vivo-generated 5,6-dehydronorketamine metabolite. The detection by our methodology of both enolic positional hydroxylations (which are chromatographically resolved – but are reported as a single species) and measurement of consistently higher 5,6-dehydronorketamine than hydroxynorketamine levels (Figs 3 and 4) lend further support to this idea and underscore some limitations of the LC-MS/MS methodology that could result in an overestimation of the quantity of 5,6-dehydronorketamine formed during biotransformation.

Independent of the possible analytical bias toward the production of 5,6-dehydronorketamine, the LC-MS/MS technique allowed us for the first time to describe the complete metabolic cascade of ketamine in the horse (Figs 2 and 4; Tables 3 and 4). Already in the first blood sample drawn after starting the ketamine infusion (i.e., at 9 min) all three metabolites (norketamine, hydroxynorketamine, and 5,6-dehydronorketamine) were detected. During the initial loading dose infusion of ketamine, norketamine was the predominant metabolite detected, however, by the time the infusion was changed to a continuous infusion at 1.5 mg/kg/h, hydroxynorketamine and 5,6-dehydronorketamine had become the predominant metabolites detected in plasma, with concentrations rapidly surmounting plasma concentrations of the parent compound ketamine and metabolite norketamine. These findings coincide with previous data in the horse, demonstrating the detection of norketamine in plasma as early as 2-5 min and peaking at 10-20 min following IV ketamine bolus administration.[34,49] Rapid metabolism of ketamine to norketamine and 5,6-dehydronorketamine has also been described in other species including sheep,[53] dog,[44] and cat.[45]

In order to better determine the rate at which norketamine and other metabolites are formed, we administered in one additional adult horse an IV bolus of 1 mg/kg ketamine over a period of 10 min and collected blood samples at 1, 2, 4 and 8 min and extended the collection period to 12 and 24 h post-ketamine administration. Plasma concentrations of norketamine were quantifiable at the 1-min sampling period and of hydroxynorketamine and 5,6-dehydronorketamine at the 2-min sampling period (Fig. 5). Given these observations and the predicted distribution

half-life ($t_{1/2\alpha}$) of ketamine of 2.34 min (Table 3), we can conclude that the conversion of ketamine to norketamine must start within compartment C_1, the plasma/extracellular fluid space (Fig. 2). As for ketamine, the plasma concentration-time curve for norketamine also declined in a biexponential fashion, suggesting the use of a similar two-compartment model for norketamine, with C_3 representing the central and C_4 the peripheral compartment of distribution. Individual distribution half-lives ($t_{1/2\alpha}$; $t_{1/2\beta}$) and AUC_0^{610} values, however, were quite different between ketamine and its first metabolite (Table 2). The model assumes that norketamine rapidly distributes to compartment C_3, which is thought to be of equal volume size as C_1, and then interchanges with compartment C_4. The median inter-compartmental transfer constant $k_{1,3}$ was 0.235 min, indicating how rapidly ketamine is metabolized to norketamine. Our model suggests that norketamine is eliminated both by conversion to hydroxynorketamine and 5,6-dehydronorketamine and by renal excretion of the intact metabolite.[50]

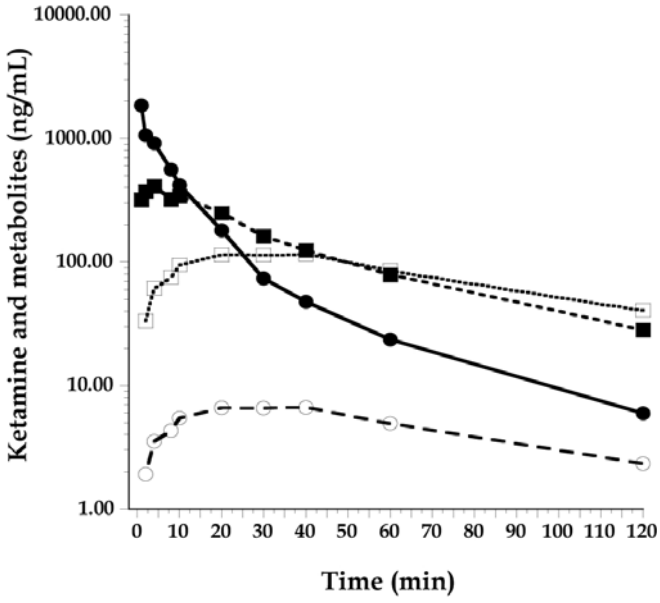

Figure 5. Plasma concentrations (0 to 120 min) of ketamine (●—), norketamine (■---), hydroxynorketamine (○— — —), and 5,6-dehydronorketamine (□▪▪▪▪▪) following a single IV administration of ketamine (1 mg/kg) in one horse.

In developing our six-compartment model to describe the distribution and metabolism of ketamine and its metabolites, we invoked the principle of parsimony. By this, we mean that we employed the minimum number of compartments, rate constants and assumptions that were necessary to describe all of the main features of the available experimental data and be consistent with the known pharmacokinetics ketamine. Our model does describe all the main features evident in the disposition curves of ketamine, norketamine, hydroxynorketamine and 5,6-dehydronorketamine during and following ketamine infusion, and R^2 values for ketamine and all metabolites in all individual horses were in excess of 0.90. The robustness of our model is further evidenced by the fact that it could well describe the pattern of the disposition curves of ketamine, norketamine, hydroxynorketamine and 5,6-dehydronorketamine following a short-term (10 min) infusion of ketamine (Fig. 5).

Despite the general success of our model, it must be acknowledged that during the period between 1 and 6 h into the continuous rate infusion our model tends to under-predict the concentrations of ketamine and norketamine. There may be several reasons for this under-prediction. First, in compartmental modeling of drug kinetics, it is generally assumed that when a drug is injected or infused into a compartment (generally the blood), it instantaneously disperses completely throughout that compartment. In reality, thorough mixing of drug throughout blood and extracellular fluid may take more than 1 min. Thus, immediately after a bolus injection or during continuous infusion of drugs, plasma concentrations of the drug will necessarily be higher than predicted by models. Secondly, in our model we assumed that the initial volumes of distribution for ketamine (C_1), norketamine (C_3), hydroxynorketamine (C_5) and 5,6-dehydronorketamine (C_7) are all equivalent. The general success of our modeling would suggest that this assumption is a good approximation. However, if this assumption is not true, then this could account for some of the discrepancies that we allude to. In support of this possibility, it is known that the metabolites of ketamine, especially hydroxynorketamine and 5,6-dehydronorketamine are more hydrophilic than ketamine and consequently their initial volumes of distribution may be somewhat larger than that of ketamine.[37,51] The third possibility that we must consider is that there could exist pathways of distribution and metabolism that we have not modeled. For example, one may contemplate the possibility that some norketamine, hydroxynorketamine or 5,6-dehydronorketamine may be reconverted to ketamine. Another possibility is that the metabolism of norketamine might also occur from C_4.[37,51]

The compartmental model presented here provides a complete conceptualisation of the distribution and metabolism of ketamine and its metabolites. Furthermore, the model provides a means of making predictions of various regimens of ketamine administration and serves as a basis on which to make additional hypotheses regarding the metabolism of ketamine. We suggest that the model could be tested by further experimentation. For example, our assumption that there is no catabolism of norketamine to ketamine could be tested by an experiment in which norketamine is injected into the blood. The disposition of both ketamine and norketamine can be described by two-compartment models, but the disposition of the more watersoluble metabolites hydroxynorketamine and 5,6-dehydronorketamine can be described by just single exponential curves. Thus, we can speculate that compartments C_2 and C_4 may represent the sequestration of ketamine and norketamine, respectively within fatty tissue.

The contribution of ketamine's metabolites to the pharmacological effects of ketamine in the horse is unknown. Studies in sheep and humans suggest that norketamine still exhibits up to 10% of the anaesthetic activity of the parent compound, while 5,6-dehydronorketamine has only about 1% of that activity.[37,53] Whether these metabolites have also analgesic or anti-inflammatory effects cannot be answered at this time.

In summary, a CRI of 1.5 mg/kg/h ketamine can safely be administered to healthy conscious horses for at least 6 h, although a slight modification of the initial infusion rate regimen may be indicated. Future experiments have to reveal the safety and efficacy of long-term ketamine administration in equine patients suffering from severe pain and/or systemic inflammatory disease and the importance of ketamine's very rapid biotransformation to norketamine, hydroxynorketamine and 5,6-dehydronorketamine in those patients.

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