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Blood–brain equilibration kinetics of levo-α-acetyl-methadol using a chronically instrumented sheep preparation

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1 The delayed onset and long duration of action of the opioid agonist levo-α-acetyl-methadol (LAAM) has been attributed to the formation of active metabolites. However, at present, little is known about the time course of blood–brain equilibration of LAAM itself.

2 The cerebral kinetics of LAAM were quantified using physiologically based kinetic models and a conscious chronically instrumented sheep preparation. Seven sheep were administered 4 min intravenous infusions of 30 mg LAAM. Concentrations of LAAM and *N*-demethylated metabolites (nor-LAAM and di-nor-LAAM) in whole blood (0–75 min) were measured using a validated HPLC assay.

3 LAAM did not alter cerebral blood flow, mean arterial pressure or cause significant respiratory depression. Cardiac output was similar to baseline at 4 min, but decreased by 30% at 10 min and remained at this level for the duration of the 75 min study period.

4 Cerebral kinetics were best described by a membrane-limited model, with a relatively slow blood–brain tissue equilibration half-life of 22 min due to intermediate permeability (56 ml min^{-1}) and a large cerebral distribution volume (724 ml).

5 In conclusion, pharmacokinetic–pharmacodynamic modelling of LAAM should account for the large equilibration delay between brain and blood caused by slow equilibration kinetics. This may account for some of the delay in onset of effect previously attributed to the delayed appearance of active metabolites in blood.
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Abbreviations: BBB, blood–brain barrier; CBF, cerebral blood flow; CO, cardiac output; di-nor-LAAM, di-*N*-desmethyl-L-α-acetyl-methadol; HPLC, high-performance liquid chromatography; LAAM, levo-α-acetyl-methadol; MAP, mean arterial pressure; nor-LAAM, *N*-desmethyl-L-α-acetyl-methadol

Introduction

Since 1965 (Dole & Nyswander, 1965), methadone substitution therapy has been the mainstay for pharmacological treatment of opioid dependence. However, up to 30% of patients experience withdrawal symptoms towards the end of an interdosing (once daily) interval (Dyer & White, 1997). Levo-α-acetyl-methadol (LAAM) is a structural analogue of methadone, and was approved in 1993 as an alternative to methadone in the U.S. (Kreek & Vocci, 2002). LAAM has a markedly longer duration of action compared to methadone, only requiring 2- to 3-day dosing intervals. A recent unblinded crossover study demonstrated that bidaily LAAM significantly attenuated withdrawal symptoms in patients who experienced significant clinical withdrawal towards the end of the once-daily methadone dosing interval (Newcombe *et al*., 2004). Similarly, patients who did not experience withdrawal symptoms while on once-daily methadone were also well controlled with bidaily LAAM.

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It is well established that the mono-*N*-desmethyl and di-*N*-desmethyl metabolites nor-LAAM and di-nor-LAAM, respectively, are much more potent than the parent drug in mediating opioid-like effects (Nickander *et al*., 1974; Smits, 1974) by having 31- and 13-fold higher affinity, respectively, for human μ-opioid receptors (Kharasch *et al*., 2005) and are eliminated more slowly (Walsh *et al*., 1998; Kharasch *et al*., 2005). This has led to the common belief that the delayed and prolonged activity of LAAM is due solely to the formation of nor- and di-nor-LAAM (Kaiko & Inturrisi, 1975; Henderson *et al*., 1976; 1977).

Recent pharmacokinetic–pharmacodynamic studies of LAAM in human volunteers have provided evidence that the activity of LAAM may not be so simply explained. However, the authors concluded that the complex nature of the LAAM concentration–effect relationship requires further investigation (Walsh *et al*., 1998; Kharasch *et al*., 2005). This might be achieved through a better understanding of the blood–brain equilibration kinetics of LAAM. Somewhat surprisingly, there

is a paucity of data regarding the rate of blood–brain partitioning of LAAM. However, it is known that LAAM has a high affinity for brain tissue as brain tissue concentrations are several fold higher than in the plasma (McMahon *et al*., 1965; Misra *et al*., 1978; Mulé & Misra, 1978).

The aim of the present study was therefore to determine the cerebral kinetics of LAAM after a short intravenous (i.v.) infusion in conscious chronically instrumented sheep. Such information would increase understanding of the factors dictating the delay between LAAM administration and its peak concentration in the brain, and how this might contribute to its delayed and prolonged duration of action.

Methods

Animal preparation

Sheep were chosen as an experimental animal as the relative perfusion of their brain and the control of cerebral blood flow (CBF) are similar to that in humans (Upton *et al*., 1994; Doolette *et al*., 1999). All experimental procedures were approved by the Animal Ethics Committee of the University of Adelaide and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The surgical procedures for preparing the sheep have been described previously (Upton *et al*., 1997). Briefly, adult female Merino sheep (approximately 50 kg) were each anaesthetized with 1.5 g, i.v., thiopentone (Abbott Australia, North Ryde, NSW, Australia) induction, 1.5% halothane (AstraZeneca Australia, North Ryde, NSW, Australia) in oxygen maintenance and instrumented as follows: catheters were chronically implanted *via* the femoral vessels in the abdominal aorta (for sampling of arterial blood), in the right atrium (for drug administration), in the pulmonary artery (for blood sampling and thermodilution measurement of cardiac output (CO)) and in the dorsal sagittal sinus (the appropriate site for sampling cerebral venous blood in sheep) (Doolette *et al*., 1999). A Doppler transducer was placed over the sagittal sinus using a previously validated method to provide an index of cerebral blood flow (Upton *et al*., 1994; Doolette *et al*., 1999). After recovery from anaesthesia, sheep were housed in metabolic crates and their catheters maintained with a saline/heparin lock (0.9%/50 i.u. ml^{-1}), with free access to food and water, under a 12 h light–dark cycle.

Study design

Seven animals were studied between 3 and 7 days after surgery. On each experimental day, sheep were placed in slings inside their metabolic crate and were prepared for physiological measurements and blood sampling. After a period of baseline measurements, LAAM (30 mg, L-α-acetyl-methadol hydrochloride; Ultrafine Chemicals, Manchester, U.K.) was administered as a 4 min constant rate infusion. For each study, the following were collected.

Blood sampling protocol

Prior to the LAAM infusion, 20 ml of blood was sampled for use in the calibration curves for the quantification of LAAM and metabolites. After the start of the infusion, 1 ml arterial and sagittal sinus blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 10, 15, 20, 30, 45, 60 and 75 min. All times are referenced to the start of the infusion. Blood samples were stored at $-20^{\circ}C$ until analysis.

Cerebral blood flow, blood gas and CO measurements

Relative changes in CBF were quantified using the Doppler flow probe and a flow meter (Bioengineering, University of Iowa, Iowa, U.S.A.) connected to an analogue to digital card (Metrabyte DAS 16-G2) and a personal computer (486-based IBM compatible). CO was measured in triplicate immediately prior to the LAAM infusions, and at 4, 10, 30 and 60 min after the start of the infusions, using a thermodilution method (Runciman *et al*., 1984). The values were averaged to obtain the mean CO at each time. Mean arterial blood pressure (MAP) was recorded continuously *via* a pressure transducer on one of the arterial catheters. Recording of MAP and CBF commenced 5 min before the start of LAAM administration (baseline) and continued for an additional 75 min.

Additional arterial blood samples were taken immediately prior to the infusions, and at 4, 10 and 30 min after the start of the infusions for blood gas analysis (ABL System 625, Radiometer, Sweden). Arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) were recorded.

HPLC conditions

The HPLC system consisted of an LC-10ATvp liquid chromatograph pump (Shimadzu Corporation, Kyoto, Japan), an SIL-10ADvp autosampler (Shimadzu), an SPD-10Avp UV–VIS detector (Shimadzu) and an SCL-10Avp system controller integrator (Shimadzu). The stationary phase consisted of a 53×7 mm ALLTIMA C8 3U Rocket column (Alltech, Illinois, U.S.A.). A 10×7 mm Alltima C18 5μ precolumn (Alltech) and a Direct-Connect Universal column prefilter (4 mm and 2 μm) were positioned ahead of the column. The mobile phase comprised 23% acetonitrile, 50 mM NaH_2PO_4 and 0.2% triethylamine, with the pH adjusted to 3.0 with orthophosphoric acid, and pumped through the system at 3.0 ml min^{-1} and monitored at 210 nm. The retention times for di-nor-LAAM, nor-LAAM, and LAAM and nor-dextropropoxyphene (internal standard) were 18.7, 23.9, and 27.7 and 13.1 min, respectively. All chemicals were of analytical grade or better.

Sample preparation

Whole blood was assayed for di-nor-LAAM, nor-LAAM and LAAM using an adaptation of Menelaou *et al*. (1999) with a calibration range of 10–2000 ng ml^{-1}. Briefly, 0.8 ml of sheep blood was mixed with 0.2 ml of MilliQ water, 50 μl internal standard (10 μg ml^{-1} nor-dextropropoxyphene in 50 mM phosphate buffer adjusted to pH 2) and 400 μl of 0.5 M sodium bicarbonate buffer adjusted to pH 9.6. Samples were then rotary mixed for 20 min with 4 ml of extraction solvent (diethylether : hexane; 30 : 70 (v v^{-1})) and centrifuged ($2000 \times g$, 10 min). The upper organic layer was transferred into new tubes containing 200 μl of 50 mM phosphate buffer adjusted to pH 2, vortexed for 45 s and centrifuged ($2000 \times g$, 10 min). The upper organic layer was aspirated and 100 μl of the acid bubble was injected on to the HPLC system.

Interassay variability was monitored with quality control (QC) samples prepared in duplicate at three concentrations: low (LQC, 40 ng ml^{-1}), medium (MQC, 200 ng ml^{-1}) and high (HQC, 800 ng ml^{-1}) of all three analytes. Interassay accuracy and precision (mean ± %coefficient of variation, $n = 6$ assays) was 102 ± 2% (LQC), 100 ± 3% (MQC) and 103 ± 2% (HQC) for LAAM, 101 ± 4% (LQC), 99 ± 2% (MQC) and 102 ± 2% (HQC) for nor-LAAM and 103 ± 2% (LQC), 101 ± 2% (MQC) and 105 ± 4% (HQC) for di-nor-LAAM. Similarly, intraassay accuracy and precision (n = 6 replicate samples) was 104 ± 5% (LQC), 100 ± 2% (MQC) and 101 ± 1% (HQC) for LAAM, 100 ± 5% (LQC), 98 ± 1% (MQC) and 99 ± 1% (HQC) for nor-LAAM and 100 ± 2% (LQC), 100 ± 1% (MQC) and 101 ± 1% (HQC) for di-nor-LAAM. The assay was both precise and accurate at the limit of quantification (10 ng ml^{-1}), with intra-assay accuracy and precision ($n = 5$–6) being 112 ± 6, 106 ± 5 and 111 ± 5% for LAAM, nor-LAAM and di-nor-LAAM, respectively. No interfering peaks were seen in any samples of drug-free sheep blood. All chemicals were of analytical grade or better.

In vivo *pharmacokinetic analysis*

As the kinetic analysis was concerned with model discrimination, models were fitted to the mean concentration of all animals at each time point. This reduces the influence of random fluctuation in concentration on model fit. In general terms, hybrid modelling of kinetics was employed. Empirical forcing functions were used to represent inputs into the brain, and curve fitting of the output of the brain to physiologically realistic models used to determine model parameters (Upton *et al*., 2000). The forcing functions are essentially a device to interpolate the available data points, as solving the models require continuous functions. Provided they fit the available data, their form has no bearing on the discrimination between various models of organ kinetics. Inputs for the brain were arterial blood concentration of the drug (C_{art}, fitted to an empirical forcing function) and CBF, while the measured sagittal sinus concentrations (C_{sag}) were used as the output for estimation of the model parameters by curve fitting. Four different kinetic models (see Figure 1) were fitted to the efferent drug concentrations (Upton *et al*., 2003):

A null model that tested the hypothesis that there was no concentration gradient across the organ

$$C_{sag} = C_{art} \tag{1}$$

A single flow-limited compartment with the mass balance equation appropriate for effluent rather than tissue drug concentrations

$$V_1 \frac{dC_{sag}}{dt} = CBF\,(C_{art} - C_{sag}) \tag{2}$$

A single flow-limited compartment with an apparent first-order loss of drug representing either irreversible deep tissue distribution or metabolism

$$V_1 \frac{dC_{sag}}{dt} = CBF\,(C_{art} - C_{sag}) - PS\ C_{sag} \tag{3}$$

A two-compartment membrane-limited model with a permeability term describing distribution into a deep tissue compartment

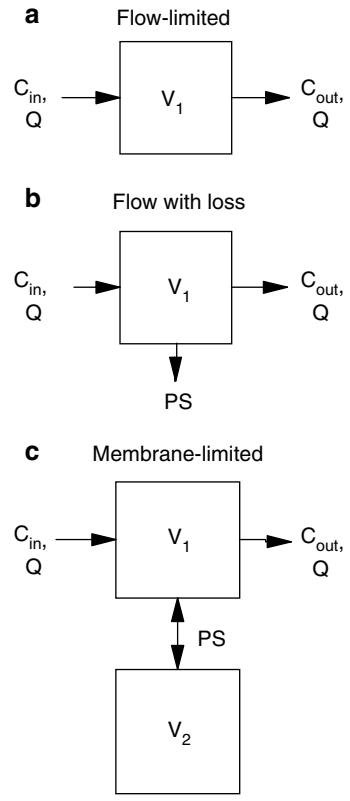

Figure 1 Graphical representation of kinetic models: flow-limited model (a, equation (2)), single flow-limited compartment with an apparent first-order loss of drug (b, equation (3)), a two-compartment membrane-limited model with a permeability term describing distribution into a deep compartment (c, equation (4)).

$$\begin{aligned} V_1 \frac{dC_{sag}}{dt} &= CBF\,(C_{art} - C_{sag}) + PS\,(C_2 - C_{sag}) \\ V_2 \frac{dC_2}{dt} &= PS\,(C_{sag} - C_2) \end{aligned} \tag{4}$$

V_1 is the volume of the first compartment of the models, CBF is the blood flow through the brain (cerebral blood flow), V_2 and C_2 are the volume of, and concentration in, the second compartment (where appropriate). The permeability–surface area coefficient (PS) is a term describing loss or exchange of drug from the first compartment. In the case of equation (3), PS represents a unidirectional loss of drug, such as metabolism or deep distribution, which is essentially irreversible. In the case of equation (4), PS represents the effective intercompartmental clearance of drug between the two compartments. Predicted apparent tissue concentrations (equivalent to C_{sag} for equations (1)–(3), C_2 for equation (4)) were also returned by the software. Fixing V_1 to 4.5 ml was tested for the two-compartment membrane-limited model, as in this model V_1 reflects the nominal vascular space of 4.5 ml in the sheep.

Using Scientist for Windows (Version 2.01, Micromath Scientific Software, Utah, U.S.A.), curve fitting employed a nonlinear least-squares regression method. Selection criteria for the final model were a statistically ($P < 0.05$) significant improvement of the fit as determined by the F-ratio test based upon the residual sum of squares, an even distribution of residuals *versus* measured concentrations and a high model

selection criterion (MSC) value (>3). The MSC of this software is essentially the Akaike Information Criterion scaled to compensate for data sets of different magnitude and has been described in detail elsewhere (Upton *et al*., 2000).

Calculated parameters

Model parameters were used to calculate secondary parameters to facilitate comparison with other opioids previously reported using this experimental preparation (Upton *et al*., 2003). Brain tissue equilibration times were calculated by using the final kinetic model and mean parameter values to simulate the time course of the brain tissue concentrations for a step increase in afferent blood LAAM concentration from 0 to 1. The times required for the brain tissue concentrations to reach 50 and 95% of the afferent blood concentrations were calculated. The former is equivalent to the half-time of equilibration only for a flow-limited (single compartment) model. The apparent permeability of the blood–brain barrier (BBB) (PS) was compared to a nominal CBF of 40 ml min^{-1} (Doolette *et al*., 1999) as has been previously determined in this experimental model. The apparent brain–blood partition coefficient (R) was calculated from V_2 and a nominal real volume of 65 ml (70% of 90 ml) for the region of the brain drained by the sagittal sinus catheter (Doolette *et al*., 1999).

The total afferent (AUC_{art}) and efferent (AUC_{sag}) area under the blood LAAM concentration–time curves to 45 min were calculated using the trapezoidal rule, using GraphPad Prism (Version 3.03, GraphPad Software, U.S.A.). AUC_{art} and AUC_{sag} were calculated from arterial and sagittal sinus blood LAAM concentrations, respectively. The retention of drug in the organ at 45 min (R%) was calculated as follows:

$$R\% = \left(1 - \frac{AUC_{out}}{AUC_{in}}\right) \times 100\% \qquad (5)$$

Drug retention represents the percentage of drug that entered the brain, but did not leave the brain by the designated time. This may be due to metabolism or deep tissue distribution from which efflux is relatively slow. The retention of drug at 45 min was selected for comparison to the structurally related opioid methadone reported in our earlier work (Foster *et al*., 2005).

Statistical analysis

GraphPad Prism (GraphPad Software) was used for all statistical analyses. In the case of CBF and MAP, which were continuously measured at 1 s intervals, a 10 s moving average from each individual animal was calculated at each of the pharmacokinetic blood sampling time points. CO, MAP, $PaCO_2$ and PaO_2 and relative changes from baseline (100%) in CBF were calculated as mean and 95% confidence intervals (CI). Statistically significant changes from base line for these pharmacodynamic data were tested using one-way repeated measures ANOVA with Bonferroni correction. An α-value of 0.05 was set for statistically significant differences. All data are reported as mean $\pm$ s.d. or mean (95% CI).

Results

Deviations from experimental protocol

In one sheep only, CBF data were unavailable due to electrical interference with the Doppler flow probe.

Pharmacodynamic data

CO remained unchanged from baseline (6.1 ± 1.1 l min^{-1}) until the end of the infusion (4 min; 6.0 ± 1.2 l min^{-1}), then steadily decreased from 10–30 min ($P<0.05$), at which time it remained stable at 30% below baseline until the end of the study (Figure 2). There were no significant changes from baseline of MAP ($P=0.38$) or CBF ($P=0.75$) at any time during the study (Figure 2). Although close to significant ($P=0.053$) at the 4 min time point only, the observed mean increase in $PaCO_2$ was approximately +3 mmHg (Figure 2). There was a corresponding increase in PaO_2 at 4 min; however, this was minor (+8 mmHg) and did not reach statistical significance ($P=0.36$).

Mild dysphoria was observed in all sheep (agitation, grinding of teeth, vocalization, nystagmus) following the LAAM infusion, which subsided by the end of the study at 75 min postdrug.

Pharmacokinetic data

Concentrations of nor-LAAM and di-nor-LAAM were below the LOQ (10 ng ml^{-1}) in all blood samples collected during the study. The peak (4 min) LAAM concentration (mean 95% CI) was 760 ng ml^{-1} (618–901 ng ml^{-1}) for arterial blood and 344 ng ml^{-1} (267–421 ng ml^{-1}) for sagittal sinus blood, which occurred at 4.5 min (Figure 3).

There was a large concentration gradient of LAAM across the brain, with the differences most obvious during the intrainfusion period (Figure 4). For the purposes of pharmacokinetic modelling, CBF was fixed at a constant value as no important time related changes were observed. A fixed value of 40 ml min^{-1} was chosen as has been previously determined as representing a baseline value in this experimental model (Doolette *et al*., 1999). In all animals, concentrations of LAAM were above the LOQ at the end of the study, although the concentration gradient across brain was minimal by approximately 45 min (Figure 4). Consequently, kinetic analysis was conducted using the entire 0–75 min concentration–time data. At 45 min, the retention (mean, 95% CI) of LAAM in the brain was 26% (22–31%). However, when the data until 75 min were included, retention dropped to 18% (13–22%).

Parameter estimates for the various models of cerebral kinetics are summarized in Table 1. The cerebral kinetics of LAAM were best described by a membrane-limited model, with the permeability term clearly best linked to a deep tissue compartment with V_1 as a fitted parameter, based on an even distribution of the residuals, higher MSC values and the F-ratio test ($P<0.00001$). Fixing V_1 to 4.5 ml was tested in the two-compartment membrane-limited model, as this V_1 reflects the nominal vascular space, which has been determined to be 4.5 ml in the sheep. The fit of the membrane-limited model to the mean sagittal sinus blood concentrations is shown in Figure 3.

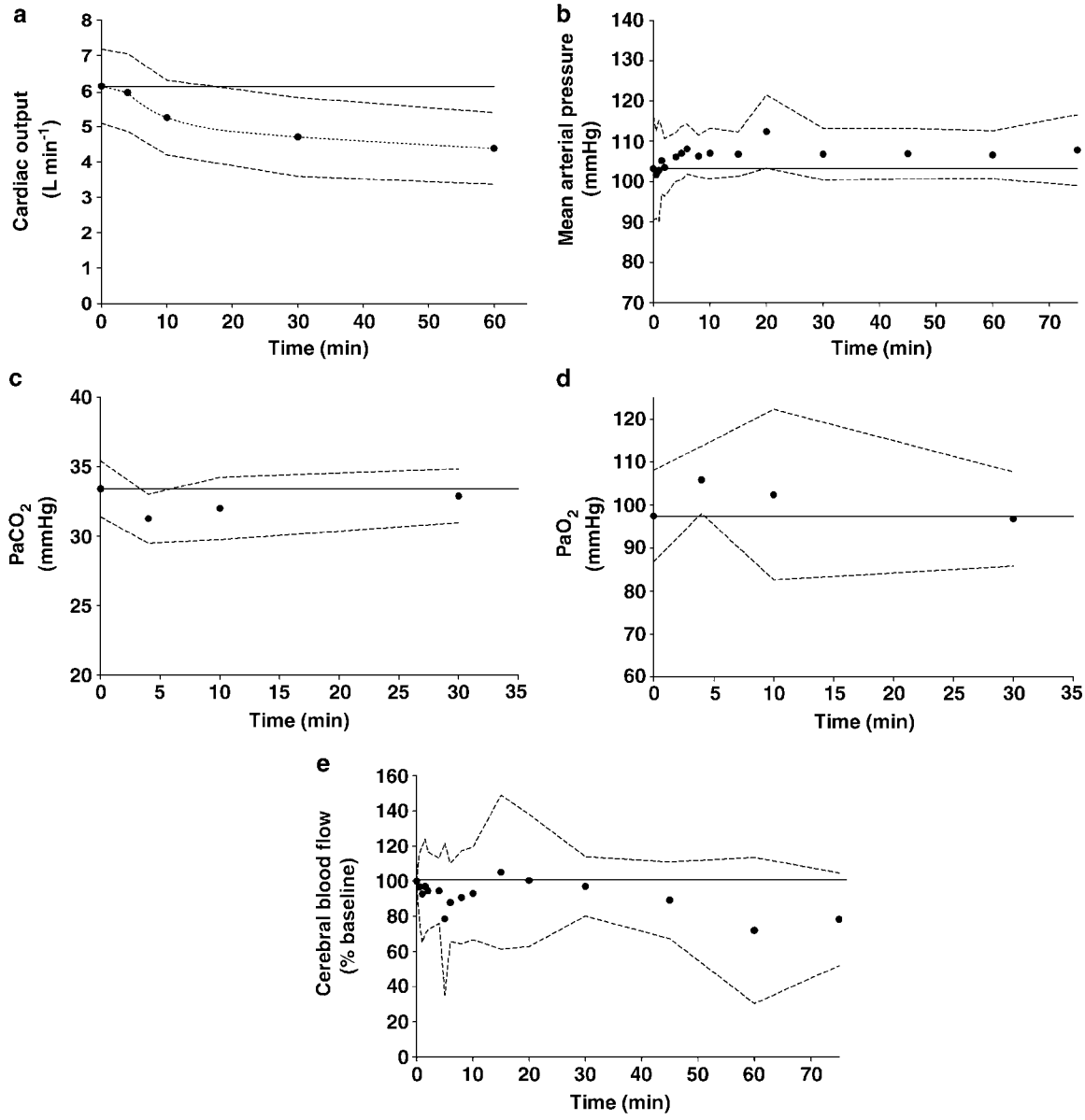

Figure 2 Mean (symbols) and 95% confidence intervals (dashed lines) of cardiac output (a), mean arterial blood pressure (b), arterial carbon dioxide tension (c), arterial oxygen tension (d) and cerebral blood flow (CBF, e) in seven sheep. Solid lines indicate the baseline value.

The value of the permeability term (PS) for LAAM was 140% of nominal CBF in the sheep. The half-life of equilibration of the initial compartment (V_1) was 0.40 min, while the times to reach 50 and 95% equilibration of the deep tissue compartment ($V_{2,brain}$) were 22 and 94 min, respectively. Assuming the nominal real brain volume drained by the sagittal sinus catheter of 65 ml, the volume of the deep tissue compartment for LAAM (724 ml) equates to an apparent blood–brain partition coefficient of 11.1.

Discussion and conclusions

Pharmacodynamic data

Despite relatively high LAAM concentrations in arterial blood ($\sim$760 ng ml^{-1}), there was no statistically significant respiratory depression. This is consistent with a lack of significant alteration of CBF, as $PaCO_2$ is a major factor influencing CBF (Michenfelder, 1990). All seven sheep developed a decrease in CO during the experimental studies. The effect was delayed, as at 4 min CO was similar to baseline, while a pronounced reduction was not observed until 10 min after the start of the infusion. CO values remained at this low level throughout the remainder of the study. This phenomenon is consistent with observations made in the dog (Waters *et al*., 1978), where a similar dose (0.3–1 mg kg^{-1}) of LAAM was given and CO did not return to baseline for 14 h after administration. One explanation could be that LAAM acts as a weak agonist at myocardial muscarinic receptors as suggested by Langley based upon binding studies (Langley *et al*., 1984; Langley, 1989), where LAAM was found to have an affinity of the same order of magnitude as pilocarpine. Furthermore, LAAM has atropine-sensitive negative chronotropic effects on the isolated

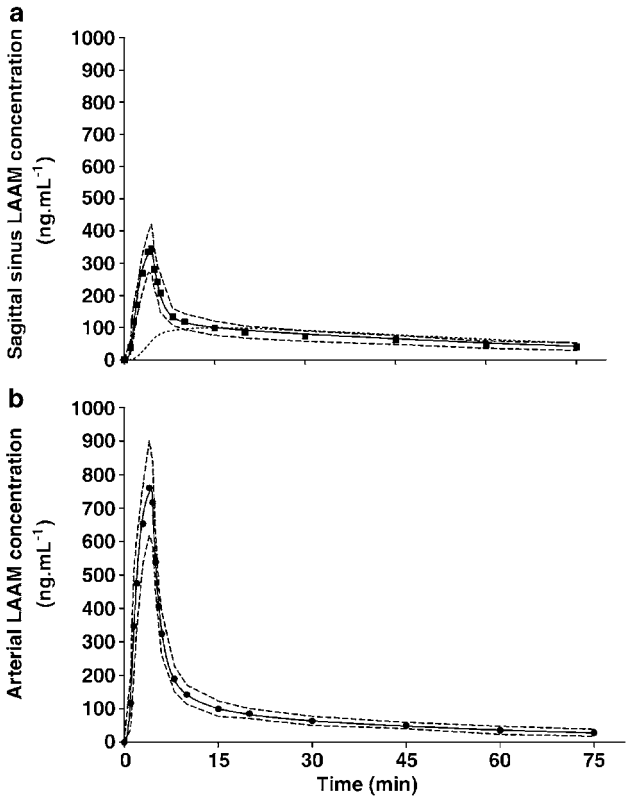

Figure 3 The measured sagittal sinus (a) and arterial (b) concentrations of LAAM in seven sheep. For arterial blood, the solid line is the fit of an empirical forcing function, which merely serves as an input to the brain. In the case of sagittal sinus blood, the solid lines are the fit of the kinetic model, which best described the data. This was the membrane-limited model with $V_{1,brain}$ as a fitted parameter (sagittal sinus; Table 1). Dotted lines represent concentrations in the deep tissue compartment based upon the final model parameters.

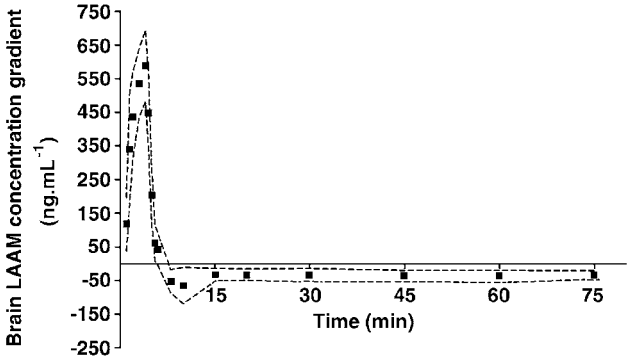

Figure 4 Mean (symbols) and 95% confidence intervals (dashed lines) of the concentration gradient across the brain (concentration in arterial blood minus concentration in sagittal sinus blood) of LAAM in seven sheep.

rat heart (Langley *et al*., 1982), and has been shown to inhibit cardiac K^+ channel currents in human cells stably transfected with the human ether-a-go-go-related potassium channel gene (Katchman *et al*., 2002; Kang *et al*., 2003). However, this does not explain the delay in effect observed here after i.v. administration, which could indicate that one of the active metabolites played a greater role than LAAM itself in depression of CO. However, we did not observe metabolites in blood samples at concentrations $>10\,ng\,ml^{-1}$, and recent work with cloned human cardiac K(+) channels (human ether-a-go-go-related gene) reports a $12\,\mu M$ IC_{50} value for nor-LAAM for the inhibition of channel current (Kang *et al*., 2003). In contrast, channel current was inhibited with a $3\,\mu M$ IC_{50} value for LAAM, which is similar to the concentrations in blood seen in our animals ($760\,ng\,ml^{-1}$, or $2.2\,\mu M$). The delay in appearance and prolonged nature of the decrease in CO may be due to an equilibration delay for LAAM between the blood and heart tissue, similar to that reported here for the brain. Whether this effect on CO in the sheep is related to reports of QTc interval prolongation in humans (Deamer *et al*., 2001), which in 2001 prompted the FDA to recommend restricted use and the EMEA to recommend alternative treatments, remains to be determined.

Cerebral distribution kinetics

We did not detect the *N*-desmethyl metabolites nor- and di-nor-LAAM in any blood samples during the study. Whether this is due to a species-dependent difference in metabolism is a possibility, although sheep are known to have high *N*-demethylation rates for various xenobiotics, and express a form of CYP3A (Danielson & Golsteyn, 1996; Galtier & Alvinerie, 1996; Dupuy *et al*., 2001), the main CY*P*450 isoform involved in the *N*-demethylation of LAAM in humans (Oda & Kharasch, 2001; Moody *et al*., 2004; Kharasch *et al*., 2005). Alternatively, it is likely that the single dose employed in our study resulted in concentrations of the metabolites that were below our limit of quantitation. In support of this after similar (20–40 mg) i.v. LAAM doses in humans, nor- and di-nor-LAAM C_{max} values were $<20\,ng\,ml^{-1}$ (Walsh *et al*., 1998), a value comparable to the LOQ of our assay.

However, we believe that the utility of our sheep preparation for the cerebral distribution kinetics of LAAM is relatively unaffected by the lack of detection of these metabolites, as it does not impact on our ability to quantitate the cerebral kinetics of LAAM itself as reported here. Indeed, if the metabolites were quantifiable, then both the kinetic modelling and its interpretation would have been more complex.

LAAM was avidly extracted across the brain, with an arteriosagittal sinus concentration gradient >50% during the infusion. We found that an average of 18% of the LAAM entering the brain had not eluted by the end of the study (75 min). Although we cannot rule out the contribution of metabolism, this would seem unlikely given the excellent description of the data by the two-compartment membrane-limited model, and comparatively poor description provided by flow-limited model with a loss term. In addition, the observed mean (95% CI) retention of LAAM in the brain at 75 min of 18% (13–22%) is in keeping with the 90 min time to reach 95% equilibration of brain tissue with blood. The retention of LAAM in the brain at 45 min (26%) was greater than that of the methadone enantiomers reported earlier (23%; Foster *et al*., 2005), most likely due to the slower cerebral equilibration of LAAM.

The volume of the initial compartment ($V_{1,brain}$) was consistently estimated to be 20–30 ml for all models tested. Fixing this volume to the nominal vascular volume of the brain in sheep (5% of 90 ml, 4.5 ml) resulted in a consistent underestimation of sagittal sinus blood concentrations of

Table 1 Goodness of fits and parameter values of the models of LAAM kinetics in the brain of seven sheep

Model	*MSC*[a]	V_1 (ml)[b]	*PS* (ml min^{-1})[b]	V_2 (ml)[b]
Null model	−1.34			
Flow	0.33	24.9 (4.3)		
Flow + loss	2.19	37.5 (11.7)	50.0 (4.2)	
Membrane limited	5.06	22.8 (2.9)	56.1 (1.7)	724.0 (58.5)
Membrane limited	3.97	4.5 (fixed)	61.1 (3.1)	606.7 (64.5)

[a]The MSC is the model selection criteria – the higher the number, the better the fit.
[b]Parameters are apparent distribution volume of the shallow compartment that includes blood (V_1), membrane permeability (PS) and deep compartment volume (V_2) in the brain. The data are shown as the mean and (s.d.) returned by the curve-fitting program.

LAAM at all timepoints after 10 min, resulting in marked increase in the residual sum of squares and a decrease in the MSC value. The value for $V_{1,brain}$ obtained for the membrane-limited model (22.8 ml) is remarkably similar to that obtained for the methadone enantiomers in this preparation (23.4 ml; Foster *et al*., 2005). While the nominal vascular space of the sheep brain is 4.5 ml, the higher fitted volume of $V_{1,brain}$ for both compounds may provide some support of our earlier hypothesis that this volume includes the vascular space between the arterial and sagittal sinus catheters in addition to the capillary space (4.5 ml). Nevertheless, the equilibration of this compartment is very rapid (0.4 min at normal CBF) and contributes relatively little to the equilibration half-life of brain tissue.

The cerebral kinetics of LAAM were extremely well described by a partially membrane limited model – the permeability term was only 40% greater than CBF. This model represents the movement of drug from an initial vascular compartment across the BBB and into the brain tissue. The relatively high membrane permeability of LAAM for the BBB was offset by a large cerebral distribution volume, resulting in a relatively long equilibration half-life of the brain tissue (22 min). This equilibration delay is very long in comparison to other opioids that we have examined in this preparation, for example, alfentanil (0.8 min; Upton *et al*., 1997), meperidine (6.3 min; Upton *et al*., 1997), fentanyl (10 min; Upton *et al*., 2004), morphine (10 min; Upton *et al*., 2003) and even methadone (17 min; Foster *et al*., 2005). The permeability of LAAM across the BBB was very similar to that of methadone reported by us in this preparation (50–60 ml min^{-1}; Foster *et al*., 2005). One explanation for this observation is their structural similarity, resulting in similar processes regulating BBB permeability including passive diffusion and active transport processes. Methadone is known to be a substrate for the efflux transporter p-glycoprotein, the gene for which is present in the sheep (Longley *et al*., 1999). However, it is currently unknown if LAAM is a substrate for this transporter.

The limited data to date suggests that LAAM has a high affinity for brain tissue as brain tissue concentrations are several fold higher than in the plasma. Brain tissue LAAM concentrations (per gram of tissue) were >20-fold higher than cerebrospinal fluid concentrations (per ml of fluid) in monkeys administered 2 mg kg^{-1} of LAAM orally (Misra *et al*., 1978). The apparent brain–blood partition coefficient (11.1), and brain tissue distribution volume (724 ml) for LAAM were greater than for (*R*)-methadone (8.2 and 531 ml, respectively) and (*S*)-methadone (9.2 and 597 ml, respectively; Foster *et al*., 2005). This is most likely due to the greater lipophilicity of LAAM (Log *P* 3.10) compared to methadone (Log *P* 2.06; Kaufmann *et al*., 1975). In the rat, McMahon *et al*. (1965) examined the tissue distribution of LAAM and nor-LAAM after a 2.5 mg kg^{-1} i.v. dose of ^{14}C LAAM or nor-LAAM. After LAAM administration, total radioactivity in the brain was two-fold greater than in plasma at 5 min, and appeared to be reasonably equal by 2 h. In contrast, when nor-LAAM was administered, brain and plasma radioactivity appeared to be reasonably equal at 5 min. Furthermore, using thin-layer chromatography to separate LAAM and nor-LAAM, the relative concentration (mean ± s.e.m.) of LAAM ($0.70 \pm 0.10\,\mu g\,g^{-1}$ tissue) in the brain was much greater than nor-LAAM ($0.11 \pm 0.05\,\mu g\,g^{-1}$ tissue) at 0.5 h after LAAM administration. The ratio of LAAM to nor-LAAM concentration remained at approximately 6 at 2 h, and reduced to approximately 2 at 4 h postdose. Collectively these albeit limited data suggest that unchanged LAAM is distributed into the brain to a much larger extent than its nor-LAAM metabolite.

It is important to note that we have described the brain tissue as a 'lumped' compartment, without differentiating the various subregions of the brain in which opioid receptors may vary in density. Such analyses are beyond the scope of the present work, as our methods assess the global brain concentrations of the drug. The concentrations in subregions of the brain (such as the respiratory centre) are assumed to vary in proportion to the global brain concentration. In support of this, concentrations of LAAM (and metabolites) in 10 different brain regions were very similar in monkeys after receiving a 2 mg kg^{-1} LAAM orally, in either acute or chronic doses, at both 6 and 24 h postdose (Misra *et al*., 1978). It could also be considered that the use of cerebral venous (e.g. sagittal sinus) blood samples is an indirect method of inferring cerebral kinetics. We have studied many drugs using this method, and have found that the estimates of PS and distribution volume in the brain are unique for each drug, and are in accord with the known physicochemical properties of the drugs. However, we have not yet compared the permeability estimates derived from our method with others such as the brain uptake index or isolated perfused brain preparations. Presently, there does not appear to be sufficient data in the literature to do so, although this would be a valuable and instructive project that could be pursued in the future.

Despite earlier reports that LAAM was simply a prodrug for its active metabolites, LAAM is now thought to contribute to the pharmacodynamic effects seen after administration of the drug (Walsh *et al*., 1998; Kharasch *et al*., 2005). When an integrated pharmacokinetic–pharmacodynamic model was fit to the data, both LAAM and

nor-LAAM concentrations adequately described the miotic effect, although the best fit was obtained with the later compound (Kharasch *et al*., 2005). Surprisingly, an effect compartment model to explain the initial delay in onset of effect did not improve model fit, and the data did not support a model which included a composite effect of all three compounds (Kharasch *et al*., 2005). The authors concluded that the complex nature of the LAAM concentration–effect relationship remains incompletely understood. Our study provides evidence that unchanged LAAM has a very slow blood–brain equilibration rate, which would be expected to be accounted for by an effect compartment model in pharmacokinetic–pharmacodynamic modelling. However, Kharasch *et al*. (2005) sampled venous blood, which will decrease the observed blood–brain equilibration delay compared to the arterial blood sampling employed in the present study. In addition, as discussed above, it appears that the metabolites of LAAM are much more restricted in their entry into the brain which may offset any potency differences, such that the relative 'effective' concentration at the site of action (the brain) is comparable. When the combined information presented above is combined with the present data showing the very slow blood–brain equilibration of LAAM, it is likely that a substantial proportion of the delay in onset of activity is due to the slow entry of the unchanged parent drug when both blood and brain concentrations of the metabolites are low. In contrast, the prolonged duration of activity is likely to arise from the sustained high concentrations of the active *N*-demethylated metabolites in the blood. Further studies examining the blood–brain equilibration of nor-LAAM and di-nor-LAAM are required to build upon the understanding of the complex pharmacokinetic–pharmacodynamic profile of LAAM.

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