

Perfusion–diffusion compartmental models describe cerebral helium kinetics at high and low cerebral blood flows in sheep

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This study evaluated the relative importance of perfusion and diffusion mechanisms in compartmental models of blood:tissue helium exchange in the brain. Helium has different physiochemical properties from previously studied gases, and is a common diluent gas in underwater diving where decompression schedules are based on theoretical models of inert gas kinetics. Helium kinetics across the cerebrum were determined during and after 15 min of helium inhalation, at separate low and high steady states of cerebral blood flow in seven sheep under isoflurane anaesthesia. Helium concentrations in arterial and sagittal sinus venous blood were determined using gas chromatographic analysis, and sagittal sinus blood flow was monitored continuously. Parameters and model selection criteria of various perfusion-limited or perfusion–diffusion compartmental models of the brain were estimated by simultaneous fitting of the models to the sagittal sinus helium concentrations for both blood flow states. Purely perfusion-limited models fitted the data poorly. Models that allowed a diffusion-limited exchange of helium between a perfusion-limited tissue compartment and an unperfused deep compartment provided better overall fit of the data and credible parameter estimates. Fit to the data was also improved by allowing countercurrent diffusion shunt of helium between arterial and venous blood. These results suggest a role of diffusion in blood:tissue helium equilibration in brain.

(Received 20 October 2004; accepted after revision 6 January 2005; first published online 13 January 2005)

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Fitting of kinetic models to experimental data is a useful method of examining the relative importance of factors such as perfusion and diffusion in the blood:tissue exchange of solutes. Inert gases, which we define as those that are non-ionizable and are not metabolized, are useful tools for investigating blood:tissue exchange models because their kinetics are easily analysed. Blood:tissue exchange for a variety of inert gases has been examined in the brain, but the relative importance of perfusion and diffusion mechanisms in the blood:tissue gas exchange has not been adequately resolved. Nitrous oxide, krypton, hydrogen, and xenon have received the most attention (Lassen & Klee, 1965; Brodersen *et al.* 1973; von Kummer & Herold, 1986; Doolette *et al.* 1998) because they are used as tracers for calculation of blood flow using the indirect Fick method (Kety & Schmidt, 1945; Lassen & Klee, 1965; Doolette *et al.* 1999). Helium has never been studied but is of interest for two reasons. Firstly, the relative importance of perfusion and diffusion may be determined by examining the kinetics of tracers with

differing physicochemical properties and, unlike gases previously studied, helium has both a high diffusivity and a low solubility. Secondly, helium is an important diluent used in breathing mixtures for deep sea diving. Decompression sickness, caused by bubble formation from excess dissolved gas accumulated in tissues during diving, is avoided using decompression (ascent) schedules based on theoretical models of the kinetics of helium in tissues. Some types of helium-based diving result in an unexpectedly high incidence of central nervous system decompression sickness (Shannon *et al.* 2004).

Compartmental kinetic models are widely used to describe blood:tissue solute exchange. In this context a compartment is represented by a single, time-varying concentration. Underlying this notion is the assumption that, owing to rapid diffusion, equilibration of inert gas concentration gradients across the tissue area represented by the compartment is much faster (well-mixed) than transport in and out of the compartment. Compartmental models are most useful for longer-term exposures

that are relevant to the use of inert gases as tracers for blood flow or in calculation of decompression schedules. The most simple and commonly used tissue model is the single, well-mixed compartment in which arterial–tissue inert gas concentration difference declines mono-exponentially and in which perfusion is often considered the rate-limiting process. Most blood flow and decompression schedule calculations are based on such perfusion-limited models. Parallel perfusion-limited compartments with different rate constants are used to accommodate tissue or whole-body departure from mono-exponential behaviour.

In order to satisfy a purely perfusion-limited model, inert gas in tissue and venous effluent blood must always be in equilibrium; however, both direct measurements and calculations from arterial–venous differences for nitrous oxide and krypton indicate this is not the case (Kety *et al.* 1948; Lassen & Klee, 1965; Doolette *et al.* 1998). Diffusion phenomena can explain inert gas gradients between tissue and venous effluent and departure from mono-exponential behaviour. Firstly, blood:tissue equilibration may be diffusion limited such that microscopic or macroscopic concentration gradients across tissue must be considered (Perl *et al.* 1965; Hills, 1977; Bassingthwaite & Goresky, 1984). Secondly, direct diffusion between non-capillary vessels allows arterial–venous shunt of inert gas (Brodersen *et al.* 1973). Diffusion phenomena can be incorporated in compartmental models via a diffusion-permeable membrane between compartments.

Change in tissue blood flow is a useful probe of blood:tissue solute exchange model validity. Our research group has developed a sheep model that allows examination of predominantly cerebral tissue *in situ* with manipulation and continuous monitoring of cerebral blood flow and repeated blood sampling (Upton *et al.* 1994). We have previously used this preparation to examine the cerebral kinetics of nitrous oxide (Doolette *et al.* 1998), and in this paper we examine the kinetics of the more diffusible but less soluble gas, helium. We collected kinetic data for helium across the brain at low and high steady state levels of cerebral blood flow and used these data to evaluate compartmental models of cerebral kinetics that included perfusion and diffusion. We hypothesized that compartmental models require diffusion-limited tissue equilibration and arterial–venous diffusion shunt to explain cerebral helium kinetics.

Methods

All surgical and experimental procedures were approved by the University of Adelaide and Institute of Medical and Veterinary Sciences animal ethics committees and were conducted in accordance with the *Australian Code*

of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council).

Surgical preparation

Seven healthy adult Merino ewes weighing approximately 50 kg were anaesthetized with 1.5 g i.v. thiopentone (Abbott Australia, NSW, Australia) induction, 1.5% halothane (Zeneca Pharmaceutical Australia, VIC, Australia) in oxygen maintenance, and instrumented as previously described (Upton *et al.* 1994; Zheng *et al.* 2000). Two 7-French gauge catheters were positioned in the thoracic aorta via the right femoral artery for measurement of blood pressure and for arterial blood sampling. A multilumen pulmonary artery flotation catheter was introduced via the right jugular vein and used in these experiments for intravenous drug administration. Via a craniotomy, a 20 MHz ultrasonic Doppler flow probe was placed on the sagittal sinus for measurement of an index of global cerebral blood flow and a 4-French catheter placed in the sagittal sinus for sampling of effluent blood from the cerebral hemispheres. The craniotomy was sealed with dental acrylic. Post-operative analgesia was provided using intramuscular xylazine (0.05 mg kg⁻¹) as required. On recovery from anaesthesia, the sheep were housed in metabolic crates with free access to food and water for at least two days' recovery from surgery and between experimental days.

Study design

On the experimental day the sheep were anaesthetized with 250 mg i.v. propofol (David Bull Laboratories, NSW, Australia) induction, 1.5% isoflurane (Abbott Australia, NSW, Australia) maintenance and mechanically ventilated via an endotracheal tube. Sheep were placed on their side. The closed circuit anaesthetic system was supplied with a fresh gas flow of 5 l min⁻¹ of 22% oxygen monitored at the common gas outlet (Capnomac, Datex, Helsinki, Finland) and the balance was nitrogen. Ultrasonic Doppler frequency shifts from the sagittal sinus probe were measured using a pulsed Doppler flow meter (Bioengineering, University of Iowa, Iowa City, IA, USA), and mean arterial blood pressure measured using a transducer on the arterial catheter; the analog signals were digitized at 1 Hz and recorded continuously to a microcomputer. End tidal carbon dioxide partial pressure (P_{ET,CO_2}) was monitored (Cardiicap, Datex, Helsinki, Finland) on the outlet of the endotracheal tube.

After a minimum of 45 min for the induction agent to be cleared from the blood, either a low or a high cerebral blood flow state (randomized order) was produced by either hyperventilating the sheep and reducing the P_{ET,CO_2} to a mean of 21 mmHg (s.d. = 8) or hypoventilating and increasing the P_{ET,CO_2} to 52 mmHg (s.d. = 2). Sheep

occasionally breathed spontaneously during hypercarbia. When the physiological measurements were stable, steady state values were recorded during a 5 min baseline period, then nitrogen was replaced by helium in the anaesthetic circuit fresh gas flow (no net change in oxygen or total gas flow) for 15 min.

Paired arterial and sagittal sinus blood samples for helium analysis were taken during the baseline period and then at 1, 2, 3, 4, 6, 8, 11, 15, 16, 17, 18, 19, 21, 23, 26, 30, and 35 min from the beginning of helium breathing. Additional arterial samples only were taken at 0.5 and 15.5 min. For each sample, after withdrawal of 5 ml blood to remove catheter dead space, approximately 3 ml blood was drawn over 10–15 s with a fresh 3 ml syringe, and immediately injected via a 26-gauge needle through the butyl rubber septum of a sealed, weighed, glass headspace vial of precisely 22 ml volume. Dead space blood was replaced and the catheter flushed with 5 ml of heparinized 0.9% saline. To minimize sample contamination with environmental gas 'Safti-ject' SV valves (Codan US Corp., CA, USA) that have no luer hub dead space were used on the catheters and the hubs of the syringes, and 26-gauge needles were filled with heparinized saline. The sampling apparatus was sealed inside a clear plastic bag accessed via latex wrist seals and continuously flushed with argon.

Sixty minutes after helium administration, the alternative cerebral blood flow state was produced and once P_{ET,CO_2} and cerebral blood flow were stable at the new level, the helium administration and blood sampling described above was repeated.

Arterial dispersion study

In order to validate the use of a central arterial blood sampling site, an eighth sheep was used to examine whether there was significant dispersion of helium during transit of blood through the arterial vessels. Under anaesthesia, a 7-French gauge catheter was advanced sufficient length from the femoral artery to position the tip near the aortic arch. A 4-French catheter was introduced into the carotid artery and the tip advanced towards the brain as previously described (Williams *et al.* 2001). Using the methods described above, paired carotid and central arterial blood samples were taken during 20 min helium administration and 20 min washout. The helium blood concentration–time curves from the two arterial sites were compared.

Helium blood solubility

A 10 ml blood sample from one sheep was collected into a heparinized vial then divided equally into four headspace vials and sealed. Vials were flushed via 26 gauge needles through the rubber septa with helium at an approximate flow of 20 ml min⁻¹ for 60 min and frequently agitated.

Vials without blood samples were flushed in series with the blood samples and these vials analysed to ensure complete flush of the headspace with helium. Each blood sample vial was inverted and approximately 2 ml blood was sampled through the septum using a syringe and 26-gauge needle, taking care not to introduce any gas from inside the vial into the syringe. This sample was injected into a fresh sealed, weighed headspace vial for helium analysis.

Helium analysis

Helium concentration in blood samples was analysed using a headspace gas chromatographic system comprising an 8500 series gas chromatograph with thermal conductivity detector and an HS-101 series automated headspace sampler (Perkin Elmer, Beaconsfield, UK) in-line between the carrier gas supply and column. Argon carrier gas flow was 15 ml min⁻¹. Samples were passed through a 1 m by 2 mm i.d. precolumn packed with 50% silica gel/50% activated charcoal to absorb water and CO₂, and sample gas separation was achieved on a 2 m by 2 mm i.d. stainless steel column packed with molecular sieve 5A 80/100 mesh. The reference channel of the thermal conductivity detector was also supplied with argon at 15 ml min⁻¹ via another molecular sieve 5A column. Column temperature was 75°C and detector temperature was 80°C.

Sample volume was determined from sample weight (mg), assuming a blood specific gravity of 1.03 g ml⁻¹. The headspace sample vials were left at room temperature for a minimum of 1 h (generally 3–8 h), to allow equilibration of the blood and headspace. Vial headspace was pressurized with carrier gas (246 kPa) and sampled using a timed (6 s) injection. Blood helium concentration expressed as millilitres of helium per millilitre of blood at room temperature and atmospheric pressure was estimated by comparison of blood sample headspace helium peak area with a six-point standard curve produced by injecting headspace vials with measured volumes of helium (0–25 µl) using a gas tight syringe. The mean r^2 value for the standard curves was 0.991 (s.d. = 0.009). Blood sample headspace helium peak area was adjusted for sample volume by multiplying by $\lambda_b + V_{HS}/V_b$ where λ_b is the helium Ostwald blood solubility at room temperature and V_{HS} and V_b are the volumes of the vial headspace and blood sample. Assay sensitivity was approximately 10⁻⁴ ml helium (ml blood)⁻¹.

Data analysis

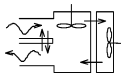
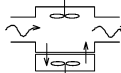
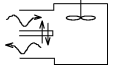
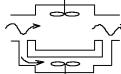
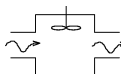
The area under the arterial and venous helium concentration–time curves was calculated by trapezoidal integration and the arterial–venous difference compared to examine recovery of helium.

A series of compartment models were used as structural models for helium kinetics in brain tissue,

Table 1. Parameters and variables in kinetic models

B_1	Fractional size of compartment 1	
c_1	Compartment 1 helium concentration	(ml ml ⁻¹)
c_2	Compartment 2 helium concentration	(ml ml ⁻¹)
c_{art}	Arterial helium concentration	(ml ml ⁻¹)
$c_{pre-cap}$	Pre-capillary (end-arterial) helium concentration	(ml ml ⁻¹)
$c_{end-cap}$	End-capillary helium concentration	(ml ml ⁻¹)
c_{ven}	Sagittal sinus helium concentration	(ml ml ⁻¹)
F_1	Fraction of cerebral blood flow directed to first compartment	
PS	Permeability surface area coefficient between compartments 1 and 2	(ml min ⁻¹)
PSC	Permeability surface area coefficient between arterial and venous blood	(ml min ⁻¹)
Q	Cerebral blood flow	(ml min ⁻¹)
V_1	Compartment 1 volume	(ml)
V_2	Compartment 2 volume	(ml)
V_{tot}	Total volume = $V_1 + V_2$	(ml)
V_{ven}	Countercurrent venous blood volume	(ml)

Table 2. Parameter estimates and MSC

Model name	Model picture	MSC (s.d.)	Parameter name	Parameter value (s.d.)
Perfusion-diffusion countercurrent		3.97 (0.67)	V_{tot}	82.5 (24.0)
			V_2	31.4 (17.3)
			PS	5.84 (3.60)
			V_{ven}	10.6 (6.0)
			PSC	6.75 (2.94)
Perfusion-diffusion base		3.82 (0.54)	V_{tot}	80.7 (16.1)
			V_2	38.9 (13.4)
			PS	7.77 (3.98)
Perfusion-limited countercurrent		3.73 (0.48)	V_{tot}	76.1 (13.0)
			V_{ven}	9.24 (6.01)
			PSC	11.3 (3.3)
Two parallel perfusion-limited		3.81 (0.51)	V_{tot}	72.4 (16.6)
			F_1	0.583 (0.105)
			B_1	0.913 (0.056)
Perfusion-limited base		3.18 (0.43)	V_{tot}	56.8 (12.6)

and were constructed as ordinary differential equations using the Scientist for Windows software package (Version 2.01, MicroMath Scientific Software, UT, USA). Parameter and variable abbreviations are defined in Table 1 and diagrammatic representations of the models are given in Table 2. The basic perfusion–diffusion model comprised two well-mixed compartments separated by a diffusion-permeable membrane; only compartment 1 is perfused with blood. Helium could potentially

bypass the ‘tissue’ compartments via arterial–venous diffusion between non-capillary vessels in a countercurrent arrangement. A constant arterial–venous helium concentration difference across the countercurrent exchange region was assumed (eqn (1)). Eqns (2) and (3) describe the perfusion–diffusion model. The potentially perfusion-limited model is the special case where $PS = 0$ and $V_2 = 0$. Venous helium concentrations were determined either from eqn (2) for the base model in which

$c_{\text{ven}} = c_{\text{end-cap}}$ or from eqn (4) in the case of the counter-current shunt model.

$$c_{\text{art}} - c_{\text{ven}} = c_{\text{pre-cap}} - c_{\text{end-cap}} \quad (1)$$

$$V_1 \times \frac{dc_{\text{end-cap}}}{dt} = Q \times (c_{\text{art}} - c_{\text{ven}}) + PS \times (c_2 - c_{\text{end-cap}}) \quad (2)$$

$$V_2 \times \frac{dc_2}{dt} = PS \times (c_{\text{end-cap}} - c_2) \quad (3)$$

$$V_{\text{ven}} \times \frac{dc_{\text{ven}}}{dt} = Q \times (c_{\text{end-cap}} - c_{\text{ven}}) + PSC \times (c_{\text{art}} - c_{\text{ven}}) \quad (4)$$

In the present models, since inert gases are freely diffusible, intravascular, extravascular, extracellular, and intracellular spaces could comprise a single compartment. A unity partition coefficient between tissue and blood ($\lambda_t (\lambda_b)^{-1} = 1$) and between compartments 1 and 2 was assumed. Additional general assumptions were that the system was linear and compartment dimensions were stationary between blood flow states.

Model inputs for each flow state were time-varying forcing functions representing the arterial blood helium concentration (c_{art}) and cerebral blood flow estimated from sagittal sinus Doppler shift (Q). These forcing functions were based on sums of exponentials or Fourier functions and selected on closest fit to the arterial or flow data.

Models were solved numerically using variable step size Adams–Moulton and backwards differentiation formula methods of the EPISODE solver for stiff and non-stiff systems (Byrne *et al.* 1977). Model parameters were estimated by fitting the model simultaneously to the observed high and low cerebral blood flow sagittal sinus concentrations by a least squares non-linear regression. Thus the models were tested for their ability to describe the change in kinetics resulting from altered flow states. Model were selected according to maximum model selection criterion (MSC), a modified Akaike information criterion calculated in Scientist for Windows:

$$\text{MSC} = \ln \left(\frac{\sum_{i=1}^n (Y_{\text{obs}_i} - \bar{Y}_{\text{obs}})^2}{\sum_{i=1}^n (Y_{\text{obs}_i} - Y_{\text{cal}_i})^2} \right) - \frac{2p}{n} \quad (5)$$

Where Y_{obs_i} , Y_{cal_i} and $\bar{Y}_{\text{obs}}$ are the observed values, the fitted values, and the mean of the observed data points, respectively, n is the number of data points, and p is the number of parameters required to obtain the fit. A large MSC indicates better model fit to the data and is penalized for model complexity (number of estimated parameters, p), and is normalized to be independent of

the scaling of data points. Candidate models were rejected if any parameters were non-identifiable, considered as a coefficient of variation of the parameter estimate greater than 100%.

In addition, nested models were compared using an extra sum of squares F -test (Draper & Smith, 1998):

$$F = \frac{(\text{RSS}_{\text{reduced}} - \text{RSS}_{\text{full}}) \times (n - p)}{\text{RSS}_{\text{full}} \times (p - q)} \quad (6)$$

Where the residual sum of squares (RSS) is equivalent to the denominator of the first term in eqn (5), p and q are the number of parameters in the full and reduced model, respectively, and a significant F -value indicates a statistically significant contribution of the additional parameter. Parameter estimates, MSC, and F -test results were calculated for each animal but are presented as the mean (s.d.) across animals. For illustration the models were also fitted to the mean data. Except where specifically indicated, statistical analysis was performed using Excel (Version 9.0. Redmond, WA, USA: Microsoft Corp, 1999).

Results

The helium Ostwald solubility coefficient in sheep blood at room temperature was 0.0077 (s.d. = 0.0004 $n = 4$) $\text{ml ml}^{-1} \text{atm}^{-1}$, slightly lower (70–93%) than published values from other species (Lango *et al.* 1996). The present lower value may be due to inaccuracy in the present method, for instance incomplete equilibration of blood and helium. Alternatively the low value may be due to the low packed cell volume of sheep blood, which is typically 35% (Hecker, 1983); helium may be highly soluble in red cell ghosts, as has been shown for other inert gases (Lango *et al.* 1996). Haematocrit measurements in the present sheep after recovery from surgery but before the experimental day ranged from 27 to 45%. Although used in the helium assay to correct for differences in sample volume, these differences in λ_b have negligible effect since V_{HS}/V_b is approximately 800 times larger than λ_b and helium partitions predominantly into the headspace. The arterial helium concentration–time curves determined simultaneously from carotid and aortic sampling sites were identical. This indicates that, at the precision of the present assay, blood sampled from any site in the arterial tree could be used as arterial concentration model input.

Input data

Although the uncalibrated Doppler index of blood flow is sufficient for model input, to assist evaluation of parameter estimates, measurements were expressed as nominal flow values in ml min^{-1} . These were calculated by recording Doppler shift at $P_{\text{ET},\text{CO}_2} = 39$ mmHg and assuming this normocarbic sagittal sinus outflow was 34 ml min^{-1} , and that Doppler shift is linear with sagittal

sinus blood flow as previously described (Upton *et al.* 1994). For fitting of models to individual animal data sets, fluctuations in flow were described by Fourier functions; however, the mean cerebral blood flow in the low flow state was 19 ml min^{-1} (s.d. = 3) and in the high flow state was 65 ml min^{-1} (s.d. = 20), a 3.4-fold difference. Cerebral blood flow was also calculated from the arterial and venous helium concentration time curves using the indirect Fick method (Doolette *et al.* 1999) assuming a unit tissue:blood partition coefficient ($\lambda_t \lambda_b^{-1}$). Cerebral blood flow thus calculated was $24 \text{ ml (100 ml)}^{-1} \text{ min}^{-1}$ (s.d. = 6) during the low flow state and $97 \text{ ml (100 ml)}^{-1} \text{ min}^{-1}$ (s.d. = 34) during the high flow state, in close agreement with the Doppler-derived data considering the volume of brain tissue drained by the sagittal sinus is approximately

60–70 ml (Hales, 1973; Upton *et al.* 1994). There was a strong linear correlation ($r^2 = 0.95$) between the individual flow measurements by the two methods. During the low flow state, the difference in area under the arterial and venous helium concentration–time curves indicated incomplete (96%, s.d. = 3%, $P = 0.0136$, two-tailed t test) washout of helium after 20 min. However, during the high flow state, recovery was not significantly different from 100% (103%, s.d. = 5%, $P = 0.1614$, two-tailed t test). This indicates that there was no loss of helium from the system. None of the models included a term for loss of helium.

Modelling

Table 2 shows the mean values of parameter estimates that provided the best MSC for the various models fitted to the individual animal data. Generally, all models could describe the high flow state well, a low MSC was typically indicative of a poor fit to the low flow state.

Perfusion-limited models

The single perfusion-limited tissue compartment model fitted the data poorly. Figure 1 shows the mean values of the sagittal sinus helium concentrations and the predictions of the single perfusion-limited compartment model fit to these data. This model predicts faster than measured helium uptake and washout for the low flow state. Fit of this model to the low flow state alone (data not shown) was no better.

The multi-exponential decline of arterial–venous inert gas concentrations has been attributed to an uneven distribution of flow to different regions of tissue (Ohta & Farhi, 1979). The two parallel compartment model provided a good MSC. In this model the sagittal sinus helium concentration was the weighted average of effluent from each compartment based on their relative blood flows. The fit to the data was improved compared to the perfusion-limited base model in all animals, but the F -test just failed to reach significance $F = 21.8$ (s.d. = 12.9), $P = 0.052$ (s.d. = 0.1268). In this two parallel perfusion-limited compartment model, the fraction of total tissue volume occupied by each compartment was an estimated parameter, and the smaller fraction ranged from 2 to 18%; however, the parameter estimates for this model were not unique, and different sets of parameter estimates could produce a reasonable fit to the data. Fixing the two compartment volumes at 63% and 37% of total tissue (data not shown), the relative volumes of grey and white matter found in domestic sheep neocortex (Ebinger, 1975), resulted in poorer fit to the data with a mean MSC of 3.43 (s.d. = 0.48).

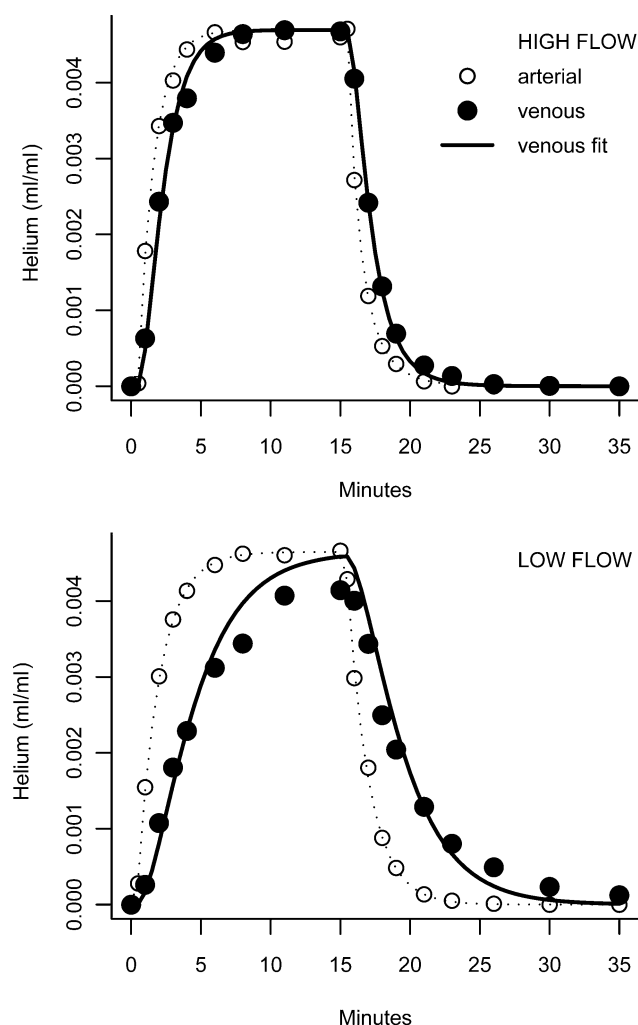


Figure 1. Perfusion-limited compartment base model fit to the mean data

Helium was administered during the interval zero to 15 min. Arterial and sagittal sinus venous blood helium concentrations (ml ml^{-1}) are shown as open and filled circles, respectively. The arterial input forcing function is shown as a dotted line. The model fit to the sagittal sinus venous blood concentrations is shown as a solid line.

Perfusion–diffusion models

The perfusion diffusion models produced a better fit to the data than the single compartment perfusion-limited models. Figure 2 shows mean sagittal sinus helium concentrations and the predictions of the base diffusion-limited model to these data. This model provides a good fit to both the high and low flow data. The perfusion–diffusion base model was a significant improvement compared to the perfusion-limited base model in all animals, and this was indicated by the substantially higher MSC and a significant F -test, $F = 22.6$ (s.d. = 12.8), $P = 0.0005$ (s.d. = 0.0009).

The estimate of the permeability surface area coefficient parameter (PS) cannot be interpreted in anatomical terms; nevertheless, the relative contribution of diffusion and perfusion can be evaluated by the ratio PS/Q where low values indicate diffusion limitation and high values indicate perfusion limitation. This quotient was 0.12 in the high flow state and 0.41 in the low flow state and indicated a mixed diffusion–perfusion limitation ($0.1 \leq \text{PS}/Q < 3$) (Piiper & Scheid, 1984).

A model comprising two parallel perfusion-limited compartments with diffusion between the compartments provided a similar fit to the data as the perfusion–diffusion base model. For most animals, the best parameter estimate was for all blood flow to one compartment, collapsing the model to the perfusion–diffusion base model. The result was a lower MSC, owing to the extra, redundant parameter, data not shown. Fixing the relative compartment volumes to represent white and grey resulted in poorer fit, data not shown.

Countercurrent shunt models

There is evidence that inert gases can bypass brain tissue by direct diffusion between non-capillary vessels (Brodersen *et al.* 1973). In the present models, countercurrent diffusion exchange of helium was allowed between arterial and venous compartments outside the main tissue compartments. Addition of a countercurrent diffusion shunt to the perfusion-limited base model resulted in a substantially improved MSC in all but animal no. 6. Parameter estimates for V_{ven} and PSC were sensitive to initial values, resulting in different fit to the data, and the estimates for V_{ven} providing the best fit were poorly identifiable with the coefficient of variation greater than 100% in four animals. Addition of a countercurrent diffusion shunt to the perfusion–diffusion base model resulted in a more modest improvement in MSC in all but animal no. 6. Although not sensitive to initial values, the estimates of V_{ven} were again poorly identifiable with the coefficient of variation greater than 100% in five animals. The ratio of the flow of helium bypassing the tissue compartment via a diffusion shunt to that delivered to the brain (PSC/Q) was calculated for the perfusion-limited

countercurrent model using the mean values as 0.17 and 0.59 in the high and low flow states, respectively. Similarly, for the perfusion–diffusion countercurrent model, the ratio of helium bypassing the tissue compartment was 0.10 and 0.36 in the high and low flow states, respectively.

Discussion

Model assumptions

In the present study all models were compartmental (lumped) models that ignored inert gas gradients that might exist owing to slow diffusion or as a result of uniform axial arrangement of capillary tissue units. The suitability of these models therefore relies on the consequence of assuming instantaneous mixing of the compartments, and this can be examined by order of magnitude comparison

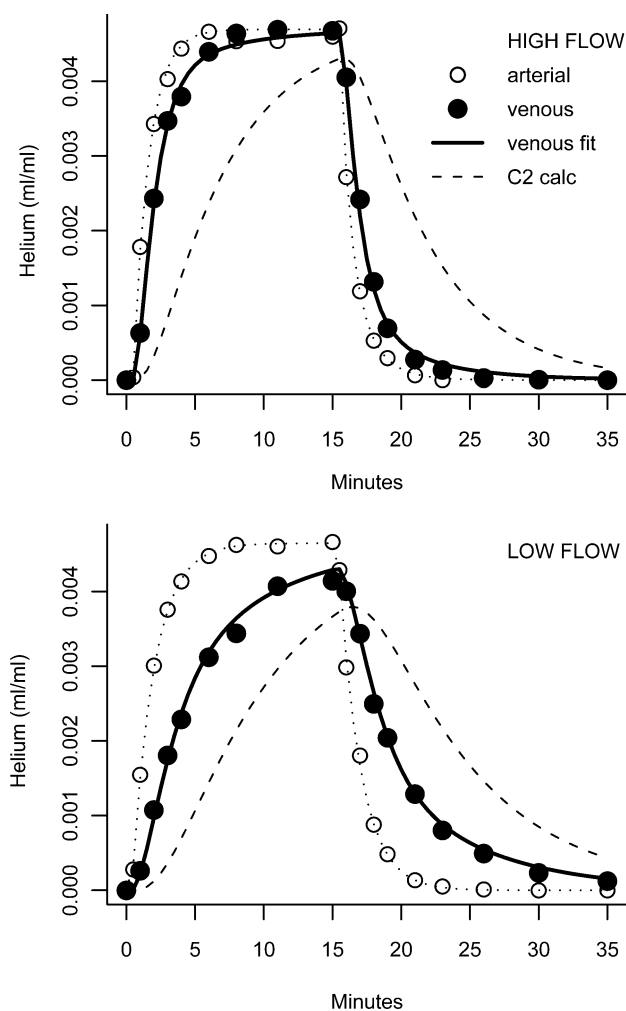


Figure 2. Perfusion–diffusion compartment base model fit to the mean data

Helium was administered during the interval zero to 15 min. Symbols and lines are as in Fig. 1 except that the calculated helium concentrations in compartment 2 are also shown as a broken line (C2 calc).

of time constants for mixing and exchange processes. Assuming values for brain of capillary tissue unit length (x) = 0.01 cm, intercapillary distance ($2r$) = 0.004 cm, diffusion coefficient (D) for helium $3.94 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, and mean capillary blood velocity (v) = 0.05 cm s^{-1} (Motti *et al.* 1986; Villringer *et al.* 1994; Lango *et al.* 1996) results in time constants for radial (perpendicular to capillary) diffusion (r^2/D) = 0.1 s, axial (parallel to capillary) diffusion (x^2/D) = 2.5 s, and capillary perfusion (x/v) = 0.2 s. Mixing to less than 1% difference occurs, by definition, in 4.6 time constants ($\ln 100/\text{rate constant}$). Radial mixing time is $\ln 100 r^2/D = 0.008 \text{ min}$. Axial mixing is dominated by the capillary perfusion such that mixing time is $\ln 100 x/v = 0.015 \text{ min}$. Both mixing times are orders of magnitude smaller than the perfusion (V_{tot}/Q or V_1/Q) and diffusion (V_2/PS) time constants estimated for the perfusion-limited and diffusion-limited models, indicating that consideration of concentration gradients across capillary tissue units is not relevant to the time course of the present studies. Unlike, for instance, muscle tissue which has a parallel arrangement of capillaries, the microcirculation of the brain is a complex reticulum with no predominant local direction of flow (Motti *et al.* 1986) which, along with rapid diffusion of helium, makes it reasonable to assume well-mixed behaviour for tissue regions larger than capillary tissue units. Indeed for tissue regions to behave as separate compartments they must be sufficiently large that the concentration gradients between adjacent regions are not equilibrated by diffusion. Such tissue regions would need to be of at least millimetre dimensions.

The assumption of stationary compartment volumes and diffusion distances between different levels of blood flow was based on the assumption that all brain capillaries are continually perfused such that changes in cerebral blood flow with hypocapnia and hypercapnia resulted in changes in capillary blood velocity, but not in recruitment of capillaries. Although there is some controversy arising from differences in histological methodologies, the literature largely supports this contention (Weiss, 1988; Villringer *et al.* 1994; Abounader *et al.* 1995).

The absolute value of the tissue volume parameter estimates relies on the values of helium tissue:blood partition coefficient and blood flow input to the models. Sagittal sinus Doppler shifts were not calibrated against flow in these animals, but there was strong agreement between Doppler estimates of blood flow based on previous calibrations and blood perfusion calculated using the indirect Fick method. This suggests the blood flow values were appropriate.

Although helium solubility in blood has been measured in several species, there are no published values of helium solubility in brain tissue (λ_t) (Lango *et al.* 1996). The muscle tissue:blood partition coefficient for helium in rats is near one, and the brain tissue:blood partition coefficients

of several inert gases (argon, nitrogen, neon, and nitrous oxide) are near one in a variety of species (rabbits, cows, and dogs), but there are no corresponding measurements in sheep (Lango *et al.* 1996). An value of one for the helium brain tissue:blood partition coefficient was assumed in the present study, to facilitate direct comparison with our previous study of nitrous oxide kinetics in the sheep brain (Doolette *et al.* 1998). Given the uncertainty in the partition coefficient, the values of total tissue volume (V_{tot}) for all models in Table 2 are reasonably consistent with the 60–70 ml measured volume of brain tissue drained by the sagittal sinus (Hales, 1973; Upton *et al.* 1994). If the value of one for the partition coefficient is an underestimate, for instance due to low helium solubility in sheep blood, the tabulated tissue volumes would be overestimates. Any downward revision of tissue volume estimates for the perfusion-limited base model become small compared to measured values.

The models are limited by the extent to which tissue kinetics can be described by arterial and venous effluent helium concentrations. The sagittal sinus blood helium concentrations represent a weighted average of concentrations from regions with different blood perfusion and the contribution of any diffusion shunt between blood vessels, but these factors are not explicitly identified. Similarly, arteriolar blood, tissue, and venular blood concentrations are inferred from the models. Possible interpretations of the model elements are considered below.

Perfusion-limited models

The single perfusion-limited tissue compartment predicts that the arterial–venous concentration difference declines mono-exponentially. There is considerable evidence against such behaviour for the brain kinetics of inert gases, for instance methane, argon (Ohta & Farhi, 1979), krypton (Lassen, 1965), hydrogen (von Kummer & Herold, 1986; von Kummer *et al.* 1986) and nitrous oxide (Doolette *et al.* 1998). The poor fit of the base perfusion-limited model to the present helium data supports these findings. Nevertheless, based on comparison of the physicochemical properties, simultaneous washout of methane and argon from dog brain has been interpreted to support perfusion-limited blood:tissue exchange (Ohta & Farhi, 1979). Two exponentials can be identified by backwards projection in such data, and are proposed to result from parallel relatively well- and poorly perfused regions (Lassen, 1965; Ohta & Farhi, 1979). A two parallel perfusion-limited compartment model with a seven-fold difference in compartmental blood perfusion fitted the helium data quite well. To behave as parallel compartments, differently perfused regions must be of sufficient size that concentration gradients between adjacent regions are not equilibrated by diffusion. The

grey and white matters are possible but unlikely candidates for such regions. Only a three-fold difference in perfusion of white and grey matters is found in sheep and other species (Hales, 1973; Klein *et al.* 1986), and a two parallel perfusion-limited compartment model with volumes representing grey and white matter fitted the helium data poorly. It may be that only two exponentials are resolved from a range of many differently perfused regions. A seven-fold range in perfusion has not been measured between different brain structures using microspheres or autoradiographic methods (Hales, 1973; Klein *et al.* 1986), but may exist at a smaller scale than resolved by these methods but still be sufficiently large to behave as a distinct compartment.

Perfusion–diffusion models

Perfusion–diffusion models provided a superior fit to the data compared with purely perfusion-limited models. In these models, the time constant for exchange of helium between the perfusion-dependent compartment and the deep compartment was not influenced by changes in blood flow. Although the model was slightly improved by inclusion of a countercurrent diffusion shunt, the base perfusion–diffusion model provided a good description of the helium cerebral kinetics at varying levels of blood flow. The same perfusion–diffusion kinetics have been found in the brain for a variety of tracers including nitrous oxide (Doolette *et al.* 1998).

The perfusion–diffusion model can be an effective first order estimate of diffusion through tissue (as for instance in a Krogh cylinder), particularly with increased equilibration time (Scheid *et al.* 1984). Here, however, the diffusion barrier was not placed at the capillary wall instead the estimated perfused compartment volume (V_1) occupied 41–64% of total tissue volume (V_{tot}). Also a radial diffusion limitation at the capillary tissue unit scale is unlikely according to the preceding time constant comparisons based on published steady state measurements of diffusivity. The diffusion-limited deep compartment can have several interpretations. It has been suggested that intracellular diffusivities are several orders of magnitude smaller than those measured on whole tissue at steady state (Hills, 1977), and the diffusion-limited deep compartment may represent the intracellular compartment. The notion of difference in intracellular and extracellular diffusivities of this magnitude is not generally supported (Bassingthwaighe & Goresky, 1984), and it is probably reasonable to combine intracellular and extracellular space into a single compartment for helium, which is highly diffusible in both aqueous and lipid solvents (Lango *et al.* 1996). Alternatively, the deep compartment may represent extracerebral brain tissue that is remote from the vascular bed drained by the sagittal sinus. In order to behave as a diffusion-limited sink and

source of helium, these regions would necessarily have lower blood perfusion than the cerebral hemispheres. The sagittal sinus does not drain the adjacent midbrain and cerebellum (Upton *et al.* 1994), but these have similar perfusion to the cerebral hemispheres (Hales, 1973). The cerebrospinal fluid has no perfusion, and although we could not find measurements of cerebrospinal fluid volume in the sheep brain, V_2 might represent total intracranial cerebrospinal fluid volume.

An alternative interpretation of the perfusion–diffusion model is that it represents diffusion between heterogeneously perfused regions. Although a model of diffusion between two perfused compartments did not provide better fit to the data than the perfusion–diffusion base model, it may be that there is insufficient information in arterial–venous data sets to discriminate between these models. The heterogeneously perfused regions may be the white and grey matter, and there is some evidence of transfer of highly diffusible hydrogen between these regions (von Kummer *et al.* 1986). Although large tissue regions may contribute to multi-exponential kinetics of inert gases, multi-exponential washout of gases is also characteristic of much smaller tissue regions. Using fine polarographic electrodes, multi-exponential hydrogen clearance curves are measured from tissue volumes with less than millimetre dimensions, and attributed to diffusion between grey white and matter (von Kummer & Herold, 1986). However, we were unable to replicate the shape of these curves using an appropriately scaled model of diffusion between grey and white matter. Also, such multi-exponential behaviour could also be due to perfusion heterogeneity, as tissue regions this small should behave as well-mixed compartments.

Countercurrent shunt

It may be that the contribution of diffusion is to allow helium to bypass the capillary exchange region. ¹³³Xenon appears in sagittal sinus blood before radio-labelled erythrocytes following simultaneous intra-arterial bolus administration (Brodersen *et al.* 1973), suggesting direct arterial–venous diffusion of gas. Although this shunt could result from a variety of vascular arrangements, it was explored formally using the countercurrent models. The parallel arrangement of centripetal arteries and centrifugal veins in the cerebral cortex (Duvernoy *et al.* 1981) may allow for such a countercurrent shunt. Countercurrent gas shunt causes a rapid convergence of venous and arterial inert gas concentrations, but delays tissue equilibration with arterial blood. Whereas tissue concentration of helium was not measured directly in the present study, analysis of nitrous oxide from samples of cerebral venous blood and tissue shows a delay of blood:tissue equilibration (Kety *et al.* 1948). Also, microprobe measurements of higher P_{CO_2} in cerebral

tissue than in local venous blood, and the reverse gradient for P_{O_2} , suggest an arterial–venous shunt (Edelman & Hoffman, 1999). The countercurrent models provided slightly larger MSC than the corresponding base models, indicating a better fit to the data, but this improvement was not statistically significant. However these models tended to provide better fit to the rapidly changing sagittal sinus helium concentrations during the first few minutes of uptake and washout. It is noteworthy that the volumes and flows and therefore time constants describing the countercurrent exchange are similar to the fast compartment of the two parallel perfusion-limited model. A small fraction (<2%) of microspheres with diameters substantially larger than brain capillaries pass through the brain (Marcus *et al.* 1976), indicating functional arteriovenous anastomoses and a small component of any arterial–venous gas shunt probably occurs via this mechanism, but was not modelled.

Comparison with previous studies

The heart appears to be the only organ across which the kinetics of helium has been studied. In that study the arterial–venous kinetics across myocardial tissue in dogs were studied following a single breath of four inert gases and analysed using a multicapillary finite element perfusion–diffusion model; that analysis indicated a diffusion shunt between capillaries for all gases and an arterial–venous diffusion shunt for helium (Wolpers *et al.* 1990). Myocardial capillaries are arranged in parallel unlike in the brain where a diffusion shunt between capillaries seems unlikely.

We have previously studied the cerebral kinetics of nitrous oxide using the same experimental preparation (Doolette *et al.* 1998) and found a similar ranking of models and parameter estimates as for helium. As was the case for helium in the present study, this previous work assumed a unit tissue:blood partition coefficient for nitrous oxide, and for comparable models the parameter estimates were similar to those found in Table 2. The model of best fit to the nitrous oxide data was the perfusion–diffusion base model; a countercurrent version was not analysed. Fitting to the mean nitrous oxide data this model returned parameter estimates $V_{\text{tot}} = 75.9$, $V_2 = 32.3$ ml, and $PS = 6.77$, using the same notation as the present work. The perfusion-limited countercurrent parameters for nitrous oxide were $V_{\text{tot}} = 60.7$, $V_{\text{ven}} = 8.31$ ml, and $PSC = 9.11$. The perfusion-limited base tissue volume estimate was 56.1 ml. For the nitrous oxide data, models comprising two parallel perfusion-limited compartments collapsed to perfusion-limited base or perfusion–diffusion base models unless the relative compartment volumes were fixed.

Both PSC and $PS = DA/h$, where D is the diffusivity of gas through the tissue and A and h are the area and

thickness of the diffusion permeable ‘membrane’ between compartments. Assuming the same diffusion geometry for the two data sets, PS should vary with D , and using the published values for skeletal muscle (Lango *et al.* 1996) would be expected to be approximately three-fold larger for helium than for nitrous oxide. Instead, the helium estimates are only 15–25% larger than the nitrous oxide estimates. There are several possibilities for the similarity of PS estimates between the two gases. Firstly, the similar time constants may describe a process that is not diffusion; however, no purely perfusion-limited models fit the nitrous oxide data well. Secondly, it may be a coincidence of the assumption of the same value of one for the partition coefficient between the compartments for both gases, and a real difference may offset the difference in diffusivity. For instance if one compartment is richer in lipid (for example white matter), the nitrous oxide partition coefficient might be quite different from the helium partition coefficient since nitrous oxide has an oil:water partition coefficient approximately twice that of helium (Lango *et al.* 1996). Finally, since the diffusivities of both gases are high, the process may not be diffusion limited *per se*. For instance the small time constant (V_{ven}/PSC) for the countercurrent diffusion process may result from complete arterial–venous concentration equilibration via rapid diffusion between only a small fraction of the arterial and venous vessels.

Conclusions

According to the models of best fit to the data, the uptake and washout of helium in the brain is determined not only by the cerebral blood flow but also by the diffusion process that may occur between heterogeneously perfused tissue regions and possibly also between non-capillary vessels. This differs from the customary assumption of perfusion-limited helium kinetics underlying decompression schedules. The implications of these data to decompression models are yet to be determined, but they show a substantially slower washout of helium from the tissue when perfusion is low than is currently recognized.

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Acknowledgements

This study was supported in part by a Jean B. Reid Medical Research Associateship, Health Science Research Committee, the University of Adelaide. We thank A. Martinez for technical assistance.