An Isolated In-Situ Rat Head Perfusion Model for Pharmacokinetic Studies

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Received July 15, 1999; accepted October 20, 1999

Purpose. To develop a viable, single pass rat head perfusion model useful for pharmacokinetic studies.

Methods. A viable rat head preparation, perfused with MOPS-buffered Ringer's solution, was developed. Radiolabelled markers (red blood cells, water and sucrose) were injected in a bolus into the internal carotid artery and collected from the posterior facial vein over 28 minutes. The double inverse Gaussian function was used to estimate the statistical moments of the markers.

Results. The viability of the perfusion was up to one hour, with optimal perfusate being 2% bovine serum albumin at 37°C, pH 7.4. The distribution volumes for red blood cells, sucrose and water (from all studies, n = 18) were 1.0 ± 0.3ml, 6.4 ± 4.2ml and 18.3 ± 11.9ml, respectively. A high normalised variance for red blood cells (3.1 ± 2.0) suggests a marked vascular heterogeneity. A higher normalised variance for water (6.4 ± 3.3) is consistent with additional diffusive/permeability limitations.

Conclusions. Analysis of the physiological parameters derived from the moments suggested that the kinetics of the markers were consistent with distribution throughout the head (weight 25g) rather than just the brain (weight 2g). This model should assist in studying solute pharmacokinetics in the head.

KEY WORDS: in-situ head perfusion; pharmacokinetics; red blood cells; water.

INTRODUCTION

The effects of drugs on tissues in the head have mainly been limited to influx studies in the brain (1,2). Sakane's group has recently explored the efflux of solutes from the brain (3). The estimation of distribution pharmacokinetic parameters in a given body region such as the head is generally difficult due to recirculation effects. The most important organ in the head is the brain, which is selectively permeable to blood-borne substances through the capillary endothelium (BBB). Techniques used to study the transport of solutes across the BBB have included indicator dilution (4), brain uptake index (1), intravenous injection (5), external registration after single injection (6), concentration profile analysis (7), isolated perfused brain (8) and in-situ perfused brain (2). Methods such as PET (9) and microdialysis (10) have also been used.

Few studies have attempted to examine the efflux parameters of solutes in the head. Of these few studies, outflow perfusate sample collections lasted for less than one minute (3,11). An underestimation of the tail of the outflow curve as a consequence may cause difficulties in accurately defining statistical moments and in modelling. We have used non-linear regression analysis, incorporating capillary permeability, tissue diffusion and vascular heterogeneity in conducting a number of pharmacokinetic studies using the single pass perfused liver and hind limb preparations both in rats (12–16) and in man (17).

The purpose of this study was to develop a single pass rat head perfusion model (including the perfusion of the right side of the brain) viable for at least one hour to allow for pharmacokinetic studies. The model has an extended viability, facilitated by (a) the introduction of a continuously oxygenated perfusate; (b) the ligation of various neck vessels supplying the contralateral side of the brain so that recirculation and the mixing of systemic blood with the oxygenated perfusate could be prevented; (c) maintaining the rat brain preparation at 37 ± 2°C; (d) monitoring systolic pressure, oxygen consumption, pH, wet/dry brain tissue weight ratio and enzyme release. The multiple indicator dilution technique and statistical moments estimation with the double inverse Gaussian distribution applied previously to other organ pharmacokinetic parameter estimation (18,19), was also used in this study.

MATERIALS AND METHODS

Surgical Procedure

Female Sprague-Dawley rats weighing 230–340g (n = 15) were fed a standard commercial diet and water ad lib. The experiments conducted in this study adhered to the "Principles of Laboratory Animal Care" and were given ethical approval by the University of Queensland Animal Ethics Committee. The rats were anaesthetised with a single i.p. injection of ketamine (8 mg/kg, Parnell Laboratories, NSW, Australia) and xylazine (10mg/kg, Bayer, NSW, Australia). Prior to arterial cannulation, heparin sodium (500 IU/kg, DBL, Australia) was injected into the femoral vein using a 27G × 1/2″ needle U-100 insulin syringe (Terumo, Japan). Arterial cannulation involved the isolation of the right carotid artery followed by the ligation of the pterygopalatine and external carotid arteries using 6-0 surgical silk (Ethicon, Australia). A 22G × 1″ i.v. catheter (Terumo, Japan) was inserted into the carotid artery, advanced into the internal carotid artery and secured by two ligatures. Immediately after arterial cannulation, blood flowed through the cannula.

Perfusion System

The perfusion was started as soon as possible through the arterial cannula with a peristaltic pump (Masterflex L/S standard drive system, Cole-Parmer, IL). The perfusion fluid consisted of 3-[N-Morpholino]propane-sulphonic acid (MOPS)-buffered Ringer's solution with a final composition of (in g/L): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, $CaCl_2.2H_2O$ 0.24, MOPS 5.23 and glucose 2. BSA (Sigma, USA) (2 or 4% w/v) was added followed by adjustment of the pH to 7.4 using HCl/NaOH. The buffer was filtered (no.541, Whatman, England) prior to use.

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ABBREVIATIONS: BSA, bovine serum albumin; BBB, blood-brain barrier; RBC, red blood cells; PET, positron emission tomography.

0724-8741/00/0200-0127$18.00/0

Initial perfusate conditions consisting of 37°C with 2% BSA were varied in order to confirm that the experiments conducted at 37°C and with 2% BSA were optimal. Variations in conditions included a lower temperature (30°C), the presence of 10% RBC in the perfusate to increase the amount of oxygen available (refer below for preparation) and higher and lower percentages of BSA (4% and 0%) in the perfusate. A flow rate of 4.6 ml/min was used (3). To enable venous cannulation, the right parotid gland was excised to expose the external jugular vein. A ligature was placed around the anterior facial vein followed by the insertion of a 20G × 1 1/4″ i.v. catheter (Terumo, Japan) into the posterior facial vein and secured with another ligature. A mixture of perfusate and blood flowed through the cannula. The superior sagittal sinus was not cannulated in this method due to the small size of the rat and the fact that the posterior facial vein was more accessible and located on the same side of the rat as the arterial catheter. Following cannulation, the left carotid artery, external and internal jugular veins were ligated to prevent recirculation and mixing of systemic blood with the perfusate. After the commencement of the perfusion, the rat was euthanased by a cardiac injection of concentrated potassium chloride.

Preparation of Blood for Perfusion

Greyhound (dog) RBC used in perfusion experiments were obtained from the University of Queensland Veterinary School during routine collections for clinical studies. Dog erythrocytes have been included in the buffer of previous perfusion studies to increase the amount of oxygen available for consumption (8,20). The blood was collected into commercial acid citrate dextrose solution and used within 24 hours of collection. The blood was centrifuged at 2500 rpm (400 × *g*) for 20 minutes (Beckman Instruments, Palo Alto, California, USA). The plasma and white cell layer were removed. The cells were then washed twice with cold sterile saline and centrifuged at the same speed for 10 minutes. The final wash was with cold MOPS buffer followed by resuspension of the RBC in the buffer and kept at 4°C overnight. On the day of the experiment, the suspension was centrifuged at 2500 rpm for 20 minutes and the supernatant removed. 100ml of the RBC were then added to 900ml of MOPS buffer for a final composition of 10% RBC in MOPS buffer.

Impulse-Response Studies

After a 20 minute stabilisation period following the start of the perfusion, 20 μl of the injectate including [^{3}H]-water (740 mBq/ml, University of Queensland, Australia) (water space marker), [^{14}C]-sucrose (250mCi/2.5ml, DuPont, Boston, Massachusetts, USA) and [^{99m}Tc]-RBC (~183mBq/ml, Royal Brisbane Hospital, Queensland, Australia; RBC were labelled with ^{99m}Technetium using an UltraTag® RBC Kit, Mallinkrodt Medical, USA) (vascular marker) and perfusate were injected as a rapid bolus via the in-flow cannula into the carotid artery. Outflow perfusate samples were collected immediately following injection in a fraction collector over 28 minutes at 1 second each (samples 1 to 20), 2.5 seconds each (samples 21 to 40), 4 seconds each (samples 41 to 70), 10 seconds each (samples 71 to 76), 30 seconds each (samples 77 to 84), 1 minute each (samples 85 to 89) and 5 minutes each (samples 90 to 92). An aliquot from each outflow sample (20μl) containing [^{3}H]-water, [^{14}C]-sucrose and [^{99m}Tc]-RBC was transferred to Eppendorf tubes for counting in a Cobra II™ Auto-Gamma® counter (Packard Instrument Co., USA). The extent of technetium decay during the process of counting was accounted for in subsequent analysis by the following equation:

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t is the number of counts in a particular sample at time t (min) and k is the first order rate constant for [^{99m}Tc] decay. After allowing the technetium in the samples to decay for at least 3 days, a further aliquot from each outflow sample (20 μl) was transferred to scintillation vials containing 5 ml Ultima-Gold™ scintillation fluid (Packard Instrument Co., USA) for beta counting in a Minaxi β Tri-Carb® 4000 series liquid scintillation counter (Packard, USA). Quenching was accounted for automatically by a built-in microprocessor after measuring the activity of an external radon source. The number of counts obtained from both β and γ samples were compared with the counts of the standards. Concentrations of [^{3}H]-water, [^{14}C]-sucrose and [^{99m}Tc]-RBC in each sample were expressed as the fraction of the dose injection per millilitre of perfusate.

Morphological Studies

Vascular Casting

Following surgery for an in-situ experiment and the stabilisation of systolic pressure (to approximately 70 mm Hg), 5mg/ml solution of Mercox (Mercox-Jap, Vilene Co., Tokyo, Japan) casting resin was made up with its catalyst and injected manually through the injection port of the arterial catheter in 3 rats. As much of the 5ml solution was injected up into the internal carotid artery to the right side of the brain as possible while maintaining the pressure at approximately 70 mm Hg. Following the injection, the entire rat was gently submersed in hot water to set the resin and then left overnight at room temperature. The head of the rat was then removed and placed in 5M sodium hydroxide for a few days to remove bone, skin and muscle from the resulting cast.

Fluoroscopic Imaging

Following the surgery for a typical in-situ experiment and the stabilisation of the systolic perfusion pressure (as mentioned above), a rat was placed under a fluoroscopy unit with image intensifier (Super 80CP, Phillips, Netherlands) and an Omnipaque solution (Sterling Pharmaceuticals, NSW) was perfused at a rate of 4.6 ml/min through the right internal carotid artery. This was continued until the medium came out of the venous catheter.

Biochemical Studies

Inflowing and outflowing perfusate samples were analysed for pO_2, pH (1312 blood gas manager, Instrumentation Laboratory, USA) and enzyme release (creatine kinase and lactate dehydrogenase) using a 747 Autoanalyser (Hitachi, Japan) after each injection. Cerebral oxygen consumption (μmol/min/g brain) was determined by the formula:

$$\text{Oxygen consumption} = \frac{(pO_{2in} - pO_{2out}) \times S \times Q}{\text{Brain wet weight (g)}} \quad (2)$$

where s = O_2 solubility (μmol L^{-1}.$Torr^{-1}$) × 0.001) and Q = flow rate (ml/min).

For experiments with 10% RBC in the perfusate, an OSM3 (Radiometer, Copenhagen) was used to determine tHb (g/L), HbO_2 (g/L) and O_2ct (μmol/L) in inflowing and outflowing perfusate samples. These values were used to derive HbO_2 consumption (μmol/min/g),

$$HbO_2 \text{ consumption} = \frac{([O_2]_{in} - [O_2]_{out}) \times Q}{\text{Brain wet weight (g)}} \times 0.44623 \quad (3)$$

where Q is the flow rate (ml/min).

The value for HbO_2 consumption was then added to the value for dissociated O_2 consumption to calculate total oxygen consumption.

Systolic pressure was monitored continuously throughout the experiment by a Transpac® transducer (Abbott, Australasia) connected to the in-flow cannula and measured using the MacLab™ Chart program (ADInstruments, USA).

At the conclusion of each experiment, the brain was removed from the skull, blotted with tissue paper to remove any blood and weighed. The brain was then placed in an oven at 70°C for at least 48 hours, after which the dry weight was determined and the wet/dry brain tissue weight ratio was able to be calculated.

Correction for Catheter Effects

Catheter experiments were also conducted in identical fashion to the brain experiments but in the absence of the organ. The catheters were joined followed by the injection of a bolus of [^{99m}Tc]-RBC and [^{3}H]-water and [^{14}C]-sucrose. Samples were collected for a period of 3 minutes after bolus injection and the data fitted by nonlinear regression (Scientist, MicroMath Scientific Software, Salt Lake City, UT) assuming an inverse Gaussian catheter function $f(t)_{cath}$ and a weighting of $1/y_{obs}$.

Empirical Transit Time Density

The outflow concentration-time curve $C_{IR}(t)$ after bolus administration of dose D into the isolated head with flow rate Q (ie. the impulse function) of a non-extracted reference solute (such as [^{99m}Tc]-RBC, [^{14}C]-sucrose and [^{3}H]-water) is related to the frequency distribution of transit times across the head $C_{IR}(t) = (D/Q)f_{cath}(t) * f_{head}(t)$ where $f_{cath}(t)$ is the catheter response and * denotes the convolution operation. The mean transit time of an indicator

$$MTT = \int_0^\infty t f_{head}(t) dt = \frac{V}{Q} \quad (4)$$

is determined by the solute's respective distribution volume in the organ V and flow Q. The variance of transit time distribution defined as

$$VTT = \int_0^\infty t^2 f(t)\, dt - MTT^2 \quad (5)$$

is used in its normalised form, called the relative dispersion of transit times

$$CV^2 = \frac{VTT}{MTT^2} \quad (6)$$

Instead of simply calculating the curve moments using Eqs. (4) and (5) by numerical integration from the measured data, a novel parametric method was applied, which has the advantages that firstly, the data can be corrected for catheter distortion without using numerical deconvolution (which is an ill-posed method), and secondly the influence of the long tail part of the curve is taken into account (reliable extrapolation to infinity). This method has been applied previously in modelling the impulse response of the isolated rat hind limb (18) and liver (19). A weighted sum of two inverse Gaussian functions was used as an empirical model of the density functions of transit times across the organ

$$f(t) = p\sqrt{\frac{MT_1}{2\pi CV_1^2 t^3}} \exp\left[-\frac{(t - MT_1)^2}{2CV_1^2 MT_1 t}\right] + (1 - p)\sqrt{\frac{MT_2}{2\pi CV_2^2 t^3}} \exp\left[-\frac{(t - MT_2)^2}{2CV_2^2 MT_2 t}\right] \quad (7)$$

where p, MT_i and CV_i^2 (i = 1,2) are independent empirical parameters used to calculate the mean transit time,

$$MTT = pMT_1 + (1 - p)MT_2 \quad (8)$$

and the variance, in its normalised form, $CV^2 = VTT/MTT^2$

$$CV^2 = \frac{p(CV_1^2 + 1)MT_1^2 + (1 - p)(CV_2^2 + 1)MT_2^2}{MTT^2} - 1 \quad (9)$$

First, the impulse response of the catheter $f_{cath}(t)$ was fitted by the same function $f(t)$ used as an empirical model. Taking the Laplace transforms $\hat{f}_{organ}(s)$ and $\hat{f}_{cath}(s)$ of $f_{organ}(t)$ and $f_{cath}(t)$, respectively, the outflow profile was then fitted by Eq. (10)

$$C_{outflow}(t) = \frac{D}{Q} L^{-1}[\hat{f}_{organ}(s)\hat{f}_{cath}(s)] \quad (10)$$

using a numerical inverse Laplace transformation algorithm ($L^{-1}[\cdot]$) in the nonlinear regression program package SCIENTIST (MicroMath Scientific Software, Salt Lake City, UT). The data were weighted according to $1/y_{obs}^2$. Note that the Laplace transforms of the functions appearing in Eq. (7) are given by

$$\hat{f}_i(s) = \exp\left\{\frac{1}{CV_i^2} - \left[\frac{MT_i}{CV_i^2/2}\left(s + \frac{1}{2MT_i\, CV_i^2}\right)\right]^{1/2}\right\} \quad (11)$$

The parameters MTT and CV^2 are directly estimated and the distribution spaces are calculated using Eq. (4). While the distribution volume, V of the indicators determined from MTT using Eq. (4) characterises the sum of their anatomical distribution spaces at steady-state, the relative dispersion of transit times across the organ (CV^2) gives information on the kinetics of distribution as shown for an organ consisting of a vascular and tissue phase (18)

$$CV^2 = CV^2_{RBC} + \frac{Q}{CL_{pT}} \frac{2\nu^2}{(1 + \nu)^2} + \frac{2}{3} \frac{d}{MTT_{RBC}} \frac{\nu}{(1 + \nu)^2} \quad (12)$$

where $MTT_{RBC} = V_{RBC}/Q$ and CV^2_{RBC} is the relative dispersion of the vascular reference (RBC). The permeation clearance, CL_{pT}, from perfusate to tissue is the effective permeability-surface area product $f_{ub}PS$. The ratio of tissue to vascular distribution volume is denoted by $\nu = V_T/V_{RBC}$ and d is a time constant which characterises the equilibration process in the tissue phase due to diffusion. Thus the total relative dispersion of an indicator consists of three terms representing the vascular convective, transmembrane permeation and tissue diffusional dispersive processes, respectively.

Statistical Analysis

All values are expressed as mean ± s.d. Results were analysed using analysis of variance (ANOVA) and Tukey with the Minitab statistical software program, version 8 (Minitab Inc., Pennsylvania, USA). The number of animals in each group of data was 3. $p < 0.05$ was accepted as the value of statistical significance.

RESULTS

Biochemical Indices

Table I shows the enzyme release (creatine kinase and lactate dehydrogenase), oxygen consumption and pH of the outflowing perfusate for all of the experiments. The level of oxygen consumption was generally consistent throughout all experimental conditions. An expected significant difference ($p < 0.001$) was observed for experiments with RBC in the perfusate compared to other perfusate conditions. Creatine kinase, a sensitive indicator of damage in the brain, was minimally released throughout each experiment. Lactate dehydrogenase, a less sensitive indicator in relation to creatine kinase also exhibited low levels in the outflowing perfusate. The pHs of the outflowing perfusate (Table I) decreased by 0.1 to 0.2 units relative to inflow pHs in all studies. This was expected since the brain utilises the oxygen in the buffer and as a result releases carbon dioxide into the venous perfusate. The presence of dissolved carbon dioxide would therefore result in the production of carbonic acid and a reduction in pH compared to the inflowing perfusate. The ratio of dry to wet brain weight was approximately 4.6 for all preparations studied (Table I).

During the bolus studies, perfusion pressure was stable at approximately 70 to 80mmHg for all experimental conditions. It remained at this level for the proceeding 50 to 60 minutes after which it increased to approximately 200mmHg. At this point, the perfusion was stopped.

Impulse Response Studies and Pharmacokinetic Modelling

Figure 1A illustrates the outflow profiles of [^{3}H]-water, [^{14}C]-sucrose and [^{99m}Tc]-RBC after a bolus injection into the rat brain 20 minutes after the start of the perfusion at 37°C with 2% BSA in the perfusate. The peak of the outflow profile for RBC was greater than those for water and sucrose. Figure 1B shows the respective fits of water, RBC and sucrose concentration-time profiles at 37°C with 2% BSA in the perfusate using the double inverse Gaussian function. In each case, the sum of the inverse Gaussians was able to aptly describe the outflow data.

Table II shows the resulting statistical moments MTT, CV^2 and volume of distribution (V) of the three solutes for the studies conducted at 37°C with 2% BSA. Water had the highest MTT and V and was significantly different to sucrose and RBC ($p < 0.01$). The CV^2 did not differ significantly between the solutes.

The experiments were then carried out with the same solutes but under different conditions in order to determine whether perfusions conducted at 37°C with 2% BSA were optimal. Table III shows the statistical moments of the three solutes estimated using the fitting of the outflow curves under the 5 different conditions with the double inverse Gaussian function and equations (7) and (8).

The MTT and volume of distribution of the RBC, water and sucrose did not differ significantly between the above 5 sets of experiments and those conducted at 37°C with 2% BSA. However, a slightly higher CV^2 for water was observed under the condition of 37°C with 0% BSA compared to the CV^2 at 30°C, 2% BSA ($p < 0.05$).

Table I. Oxygen Consumption, Enzyme Release, pH, and Wet/Dry Brain Weight Ratio During 6 Different Experimental Conditions (Values Are Presented as Mean ± s.d., n = 6[a])

Experimental condition	Oxygen consumption (μmol/min/g)	Creatine kinase (U/L)	Lactate dehydrogenase (U/L)	pH	Wet/dry brain weight ratio
30°C, 0% BSA	2.7 ± 0.3	2.7 ± 4.2	16.7 ± 11.8	7.25 ± 0.01	4.31 ± 0.38
37°C, 0% BSA	2.3 ± 0.2	13.7 ± 12.7	20.0 ± 7.4	7.26 ± 0.02	4.81 ± 0.21
30°C, 2% BSA	2.3 ± 0.6	4.5 ± 7.1	10.2 ± 19.1	7.27 ± 0.02	4.61 ± 0.02
37°C, 2% BSA	2.1 ± 0.3	11.7 ± 9.1	25 ± 19.8	7.27 ± 0.07	4.93 ± 0.13
37°C, 4% BSA	2.5 ± 0.2	1.7 ± 2.6	9.2 ± 9.2	7.25 ± 0.02	4.70 ± 0.10
37°C, 2% BSA (10% RBC)	5.8 ± 0.7[b]	9.0 ± 1.8	23.5 ± 12.2	7.17 ± 0.06[c]	4.68 ± 0.13

[a] n = 6 for all values except wet/dry brain weight ratio values which are based on n = 3.
[b] Differs significantly ($p < 0.001$) from the oxygen consumption values for other perfusate conditions.
[c] Differs significanfly ($p < 0.01$) from the pH values for other perfusate conditions.

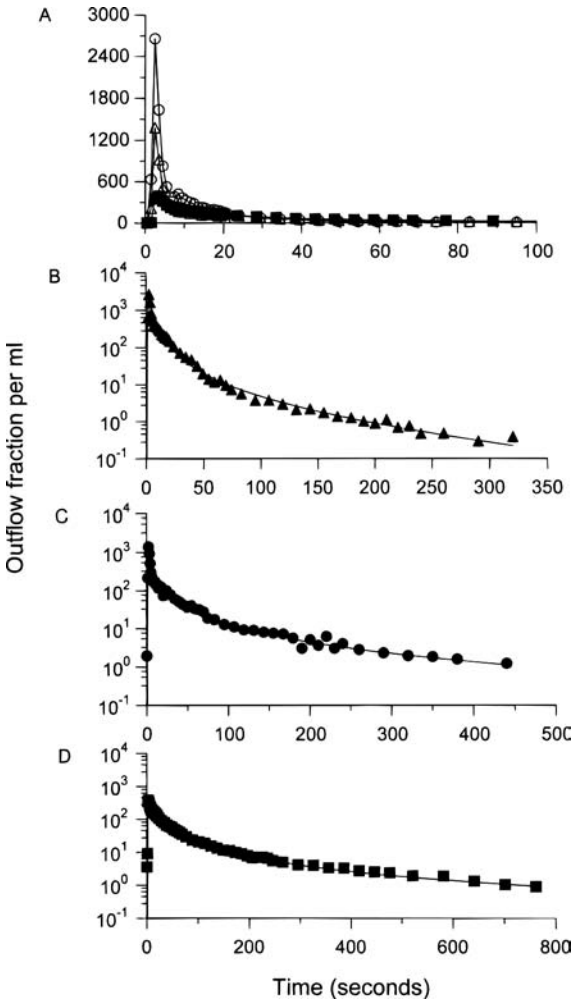

Fig. 1. (A) Representative venous outflow profiles of [^{3}H]-water (■), [^{99m}Tc]-RBC (○) and [^{14}C]-sucrose (△) from an isolated in-situ rat head perfusion experiment conducted at 37°C and with 2% BSA in the perfusate. Representative fits of venous outflow concentration-time data on a log-linear scale (y-axis values have been multiplied by 1000) for (B) [^{99m}Tc]-RBC (▲), (C) [^{14}C]-sucrose (●) and (D) [^{3}H]-water (■) from isolated in-situ rat head perfusion experiments conducted at 37°C with 2% BSA in the perfusate.

Table IV shows the amount of sucrose present in different parts of the brain on cessation of a given study. The levels were higher in the cortex, especially the right side, than elsewhere.

Table II. Statistical Moments Determined from the Double Inverse Gaussian Distribution Function Fit of Outflow Concentration-Time Data at 37°C, 2% BSA (Values Are Presented as Mean (n = 3) ± s.d.)

Solute	MTT (seconds)	CV^2	V (ml)
RBC	16.0 ± 4.1	3.9 ± 1.9	1.2 ± 0.3[b]
Sucrose	52.8 ± 23.3	3.3 ± 1.3	4.0 ± 1.6[b]
Water	239.5 ± 11.0[a]	7.8 ± 2.6	18.1 ± 0.7[a]

[a] MTT and V for water differ significantly ($p < 0.01$) from MTT and V for RBC and sucrose.
[b] significant difference between the volumes of RBC and sucrose ($p < 0.01$).

DISCUSSION

In the present study, a single pass rat head perfusion model viable for 1 hour and suitable for drug transport studies was established. The present model offers several advantages over previous *in vivo* models including the ability to control perfusate conditions (eg. by altering the perfusate composition, pH, osmolality, ionic content, protein concentration and flow rate). Further, *in vivo* models do not enable the effect of blood flow and protein binding dependence of brain pharmacokinetics to be explored to the extent possible with *in situ* perfusions, as has been demonstrated in hepatic (21) and leg (16) solute disposition. Also, *in situ* models such as the single pass model described in this work avoid the effects of hepatic and renal elimination of solutes which occurs following vascular and extravascular administration of solutes *in vivo*. In comparison to a quantitative imaging technique such as PET (9), the present in-situ model is able to differentiate between the drug in the brain and the blood since solute analysis is based on outflow perfusate samples over an extended period of time. Finally, unlike microdialysis (10), the in-situ model determines drug disposition in the whole brain, not just discrete areas of the brain.

The present single pass perfused head preparation L was developed to measure the pharmacokinetics of a range of molecules which are passively or actively transported across the BBB (as shown by water in this study), as well as poorly extracted solutes and large proteins which may or may not remain in the vascular space of the brain/head (as shown by sucrose and RBC in this study). The present preparation can be used to conduct single pass studies for up to one hour with the majority of solute uptake being into the brain since the solutes are injected directly into the internal carotid artery. A flow rate of 4.6ml/min was determined by Sakane *et al.* (3) to prevent perfusate from flowing to the left side of the brain. This result was confirmed in the present work (Figs. 2A and B). In contrast to the present method, the brain uptake index method is based on decapitation after 15 seconds (1). The *in vivo* multiple indicator dilution technique (5), has limited application due to recirculation effects. An *in situ* model developed by Preston *et al.* (22) however, is able to estimate influx in the brain over a longer time period (30 mins).

A limitation of impulse-response studies is the definition of sites and extent of uptake. Table IV shows that uptake into the brain is not necessarily uniform. It is suggested that in further development of the technique, microdialysis be used in conjunction with the present method to measure solute concentrations in extracranial parts of the head.

The brain is the largest, most sensitive to fluctuations in energy metabolism and blood flow and most important organ within the head and therefore its viability needs to be maintained in any perfused head preparation. Creatine kinase (bb) was used as a measure of cellular damage as it is found in the brain and other tissues but is not normally present in the CSF or serum. Hence, its release from the brain into these body fluids is a sensitive indicator of any damage that may be occurring (23,24). This work showed minimal creatine kinase release for the first 40 minutes of the perfusion. The oxygen consumption in the brains from our studies was also similar to the cerebral oxygen consumption of approximately 2.09 μmol/g/min reported in anaesthetised male Fisher rats (25). These results therefore suggest that the energy metabolism of the brain was preserved.

Table III. Statistical Moments Determined from the Double Inverse Gaussian Distribution Function Fit of Outflow Concentration-Time Data Under Different Perfusion Conditions (Values Are Presented as Mean (n = 3) ± s.d.)

Condition	Solute	MTT (seconds)	CV^2	V (ml)
0% BSA, 30°C	RBC	11.3 ± 2.0	2.9 ± 0.5	0.8 ± 0.2
	Water	456.6 ± 208.4	8.7 ± 3.5	33.1 ± 14.7
	Sucrose	132.7 ± 96.7	5.1 ± 2.0	9.7 ± 7.1
0% BSA, 37°C	RBC	10.8 ± 3.2	5.1 ± 4.2	0.8 ± 0.3
	Water	289.1 ± 284.1	10.1 ± 2.6[a]	20.6 ± 20.5
	Sucrose	105.9 ± 65.3	11.5 ± 10.8	7.4 ± 4.7
2% BSA, 30°C	RBC	12.2 ± 3.4	2.3 ± 0.4	0.9 ± 0.3
	Water	181.9 ± 88.6	3.0 ± 1.3[a]	13.0 ± 6.4
	Sucrose	87.4 ± 46.2	3.9 ± 0.9	6.6 ± 3.6
2% BSA, 37°C	RBC	15.7 ± 3.3	2.9 ± 1.6	1.1 ± 0.3
(10% RBC)	Water	176.1 ± 93.3	4.5 ± 1.5	12.7 ± 6.6
	Sucrose	N.D.[b]	N.D.[b]	N.D.[b]
4% BSA, 37°C	RBC	15.0 ± 2.3	1.9 ± 0.2	1.1 ± 0.1
	Water	164.1 ± 36.7	4.4 ± 1.0	12.2 ± 2.3
	Sucrose	59.0 ± 18.3	3.6 ± 1.8	4.4 ± 1.5

[a] Differ significantly ($p < 0.05$).
[b] not determined.

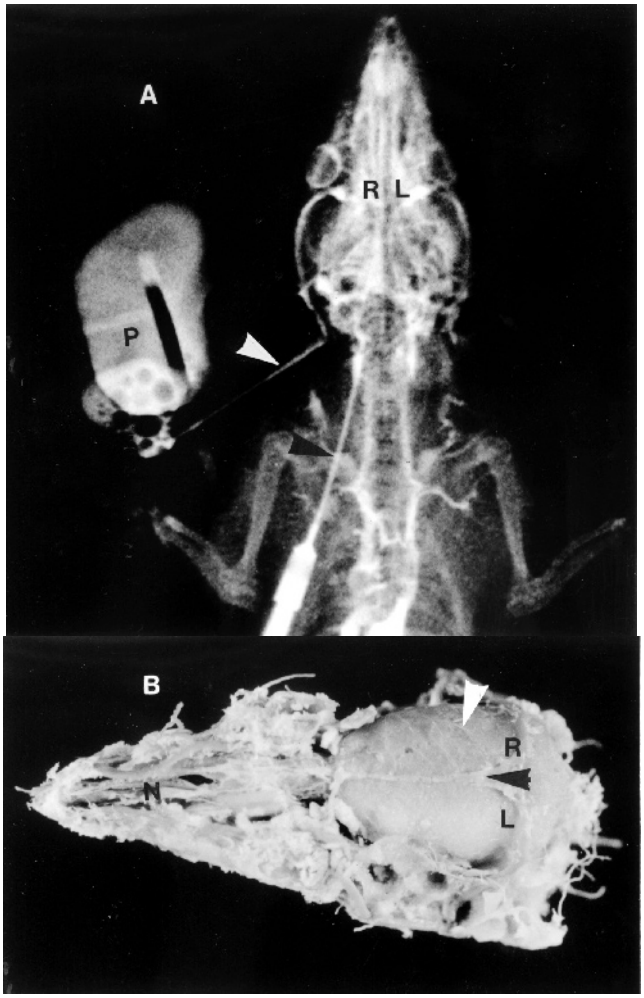

Fig. 2. (A) Fluoroscopic image of a rat in an anterior position. Radiopaque media has been perfused through the right internal carotid artery (black arrow). The media is evident in the right (R) side of the brain and to a lesser extent in the left side (L). The media is also present in other areas of the head including the nasal area as well as the eyes. The media is then seen exiting the venous catheter (white arrow) and being collected in a pool to the left of it (P). (B) A vascular cast of a rat head with the brain intact. The resin is present in the vessels of the right (R) side of the brain (white arrow) but not the left (L) and is also present in the major sinuses of both sides of the brain (black arrow) and vessels originating in the nasal area of the head (N).

Studies conducted using fluoroscopic imaging (Fig. 2A) and vascular casting (Fig. 2B), which were initiated at least 20 minutes after the start of the perfusion also showed that flow to the brain was preserved.

The distribution volumes of the three solutes investigated (Table II) are more consistent with distribution of solutes in the head than in the brain alone (average weight of head 25g; 64% water; estimated vascular volume of brain 1% (v/w) (2)). The larger vascular volume of the head was defined by both fluoroscopic imaging of the head (Fig. 2A) and vascular casting (Fig. 2B).

Fluoroscopic imaging (Fig. 2A) shows that retrograde perfusion of the vertebral arteries may account for some of the perfusate lost (approximately 7%), evident from a slightly lower outflow rate (generally 4.3–4.6 ml/min) compared to the inflow rate (4.6 ml/min). Due to the difficulty of manoeuvring the rat to locate the vertebral arteries whilst the arterial catheter had already been inserted and the perfusion commenced, the vertebral arteries were left unligated in the experiments.

The brain uptake index (26) for sucrose in the rat brain has been determined to be 1.41 ± 0.47 which indicates negligible extraction (1). The larger volumes of distribution for sucrose compared with RBC are consistent with distribution into the extracellular space of structures in the head in addition to the brain before being eluted in the venous outflow.

The distribution volume of water (18.1 ml) is 12 times the actual volume of water in the brain, (estimated to be 1.5 ml by subtracting the average dry weight of the brain from the average wet weight). A diagrammatic representation of possible sites of distribution is shown in Fig. 3. The nose and eye regions account for much of the distribution.

The CV^2 of water was consistently two-fold greater than that of RBC. Weiss and Roberts (18) have shown that the CV^2 can be increased by either permeability barriers and/or diffusion barriers. Both water and sucrose will encounter these barriers in the head accounting for the differences in the relative values of CV^2. These results are in contrast to the distribution of water, sucrose and RBC in the liver (27). The CV^2 for each of these

Table IV. Amount of [^{14}C]-Sucrose Radioactivity Remaining in Brain 30 Minutes After Injection in the In-Situ Preparation. Values Are Presented as As Mean ± s.d., n = 2.

Brain region	Estimated remaining fraction of dose injected/g brain
Left cortex	0.00051 ± 0.00044
Right cortex	0.00071 ± 0.00047
Cerebellum	0.00038 ± 0.00017

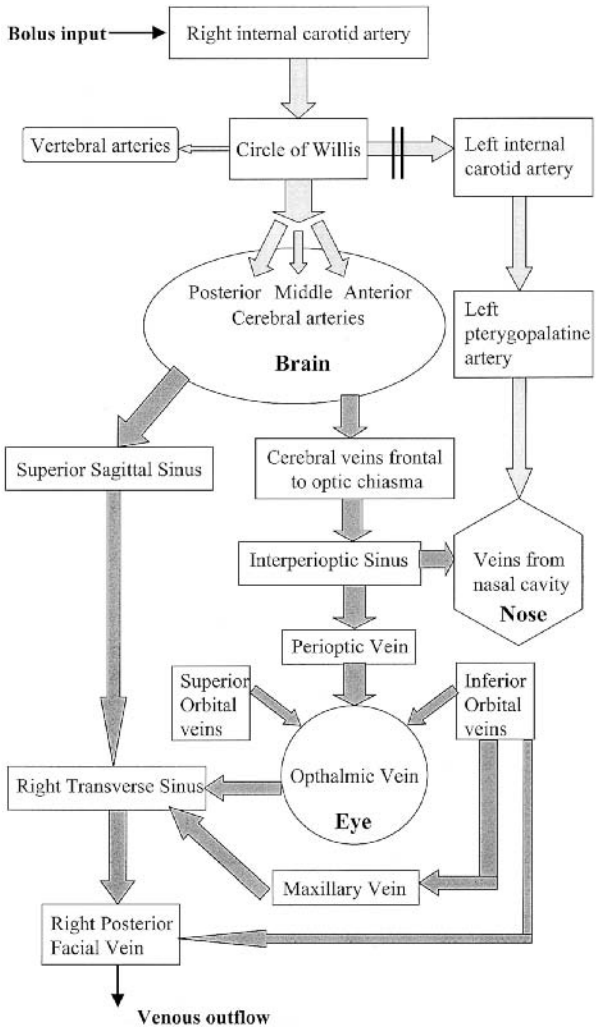

Fig. 3. Postulated path of perfusate through the head between the arterial inflow (right internal carotid artery) and the venous outflow (right posterior facial vein) in the isolated in-situ rat head preparation. Light grey and dark grey arrows denote the arterial and venous circulations of the head, respectively.

markers appears to be equal (14), consistent with the concept of a flow-limited diffusion of non-extracted solutes in the liver (28).

This study has shown that a single pass rat head perfusion model, viable for at least one hour, can be used for drug transport studies. Impulse response studies and the empirical transit time density model were used to determine the mean transit time, volume of distribution and CV^2 of reference indicators, RBC, water and sucrose. Values obtained for these three parameters during the experiments suggest that the BBB remained intact over the entire perfusion period. An important outcome from this study was to show that solutes injected in the present preparation perfused not only the brain but parts of the head as well.

ACKNOWLEDGMENTS

The authors wish to thank Dr Marjorie Green for her expertise in microsurgery and Dr Paul Mills for his assistance with the fluoroscopic imaging and vascular casting. The authors also wish to acknowledge the support of the National Health and Medical Research Council of Australia, the Queensland and New South Wales Lions Kidney and Medical Research Foundation and the Princess Alexandra Hospital Research and Development Foundation.

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