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Distribution kinetics of solutes in the isolated in-situ perfused rat head using the multiple indicator dilution technique and a physiological two-barrier model

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Abstract

The purpose of this study was to determine the pharmacokinetics of [^{14}C]diclofenac, [^{14}C]salicylate and [^{3}H]clonidine using a single pass rat head perfusion preparation. The head was perfused with 3-[N-morpholino] propane-sulfonic acid-buffered Ringer's solution. ^{99m}Tc-red blood cells and a drug were injected in a bolus into the internal carotid artery and collected from the posterior facial vein over 28 min. A two-barrier stochastic organ model was used to estimate the statistical moments of the solutes. Plasma, interstitial and cellular distribution volumes for the solutes ranged from 1.0 mL (diclofenac) to 1.6 mL (salicylate), 2.0 mL (diclofenac) to 4.2 mL (water) and 3.9 mL (salicylate) to 20.9 mL (diclofenac), respectively. A comparison of these volumes to water indicated some exclusion of the drugs from the interstitial space and salicylate from the cellular space. Permeability–surface area (PS) products calculated from plasma to interstitial fluid permeation clearances (CL_{PI}) (range 0.02–0.40 mL s^{-1}) and fractions of solute unbound in the perfusate were in the order: diclofenac > salicylate > clonidine > sucrose (from 41.8 to 0.10 mL s^{-1}). The slow efflux of diclofenac, compared with clonidine and salicylate, may be related to its low average unbound fraction in the cells. This work accounts for the tail of disposition curves in describing pharmacokinetics in the head.

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Introduction

The ability to determine the pharmacokinetics of different solutes in the head is important for a better understanding of drug/toxin distribution both in normal and in pathophysiological states. Almost all techniques developed over the past thirty years to study drug transport in the head (including significant studies conducted by Andjus et al (1967), Oldendorf (1970) and Takasato et al (1984)), have been limited mainly to estimating unidirectional influx of a solute in the brain. Only a few studies have attempted to examine influx and efflux parameters of solutes in the head (Sakane et al 1991; Foster et al 2000). In this context, the head is a heterogeneous region consisting of a number of parts including the visual and auditory systems, the olfactory region, the oral cavity and the brain.

Optimal pharmacokinetic analysis requires both early-in-time and late-in-time data. Therefore, some studies may be restricted by the small data sets associated with destructive sampling of individual animals. The single-pass perfusion or multiple indicator dilution method allows late-in-time data to be collected avoiding recirculation effects present in-vivo. To date, only two studies have been conducted in the perfused head attempting to describe the influx and efflux of various drugs

across the vascular (e.g. blood–brain barrier (BBB)) and cellular barriers using pharmacokinetic analysis and a vascular marker (Sakane et al 1991; Foster et al 2000). However, in Sakane's study, the collection of outflow perfusate samples for less than 1 min led to a gross under-estimation of the distribution volume of the preparation with the questionable conclusion that the preparation would be suitable for brain pharmacokinetic studies. In contrast, our rat head perfusion model gave more reliable results due to a much longer collection period of 28 min (Foster et al 2000).

Pharmacokinetic studies involving isolated perfused regions and pharmacokinetic analyses have been undertaken by a number of authors and include studies on organs such as the liver, kidney, heart and lung (Bassingthwaighte & Goresky 1984), and body regions such as the hind limb (Wu et al 1993; Weiss et al 1997a). With time, the models have increased in sophistication by taking into account catheter effects, intra-vascular mixing (flow heterogeneity), membrane permeation and intra-tissue diffusion. We developed a model that combined each of the determinants for solute transport in an isolated region using a stochastic approach to evaluate the transit time distributions of solutes (Weiss & Roberts 1996). The model has been used to describe the distribution kinetics of lidocaine, diazepam and antipyrine in the isolated perfused rat hind limb (Weiss et al 1997a). Also, Weiss et al (1997b, 1998) applied this general organ model in the analysis of hepatic pharmacokinetics.

In this study, we have examined drug disposition in an isolated, perfused, in-situ rat head perfusion (Foster et al 2000). We applied our stochastic model of organ and regional pharmacokinetics to determine distribution and permeability parameters for various solutes with widely differing physicochemical properties. The solutes used in this study included [^{3}H]clonidine, [^{14}C]diclofenac, [^{14}C]salicylate, [^{14}C]sucrose and [^{3}H]water with the simultaneous injection of the vascular marker [^{99m}Tc]-red blood cells (RBC). Studies were conducted by perfusing the head with 3-[N-morpholino] propanesulfonic acid (MOPS)-buffered Ringer's solution containing 2% bovine serum albumin (BSA) at 37°C.

Materials and Methods

Perfused head preparation

The experiments conducted in this study were given ethical approval by the University of Queensland Animal Ethics Committee.

The development of the perfused adult rat head model and its validation using vascular casting and fluoroscopic imaging have been described elsewhere (Foster et al 2000). In brief, female Sprague-Dawley rats (220–280 g) (n = 9) were anaesthetized with a single intraperitoneal injection of ketamine (80 mg kg^{-1}, Parnell Laboratories, NSW, Australia) and xylazine (10 mg kg^{-1}, Bayer, NSW, Australia). The right internal carotid artery was cannulated with a 22G×1″ intravenous catheter (Terumo, Japan) following the ligation of the pterygopalatine and external carotid arteries. This ensured that all in-flowing perfusate flowed to the brain first (refer to Foster et al (2000) for postulated path of perfusate between arterial inflow and venous outflow). Immediately after arterial cannulation, blood flowed through the cannula. The perfusion through the arterial cannula was started as soon as possible using a peristaltic pump (Masterflex L/S standard drive system, Cole-Parmer, IL). The rat brain and head were perfused with MOPS-buffered Ringer's solution (pH 7.4) containing 2% BSA (Sigma, USA). The in-flowing perfusate temperature was maintained at 36°C±1°C by performing the perfusion in an enclosed humidified incubator. The temperature was monitored using a temperature probe. A flow rate of 4.6 mL min^{-1} was used to minimize the flow of perfusate into the right side of the brain (Sakane et al 1991).

Venous cannulation was performed in a similar fashion to Sakane et al (1991). A 20G×1-1/4″ intravenous catheter (Terumo, Japan) was inserted into the external jugular vein and advanced as close to the posterior facial vein as possible. A mixture of perfusate and blood flowed through the cannula. 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 killed by a cardiac injection of concentrated potassium chloride.

Impulse response experiments

Clonidine, diclofenac, salicylate and sucrose were selected for this study from a range of solutes tested as they had relatively high recoveries (availability $F > 69\%$). The recovery was based on the remaining percentage of drug in the outflowing perfusate over the possible sampling time of 28 min compared with what was injected arterially. After a 20-min stabilization period, a vascular marker, [^{99m}Tc]RBC (~ 183 mBq mL^{-1}, Royal Brisbane Hospital, Queensland, Australia) and a solute, [^{3}H]clonidine (58 Ci mmol^{-1}, NEN, USA), [^{14}C]diclofenac (9.2 mBq/3 mg, Ciba Geigy, Sydney, Australia), [^{14}C]salicylate (54 mCi mmol^{-1}, NEN, USA), [^{14}C]sucrose

(442 mCi mmol^{-1}, NEN, USA) or [^{3}H]water (740 mBq mL^{-1}, University of Queensland, Australia), were injected simultaneously by bolus injection at a flow rate of 4.6 mL min^{-1} into the right internal carotid artery. RBC were labelled with ^{99m}technetium using an UltraTag RBC Kit (Mallinkrodt Medical, USA). Samples were collected immediately following the injection from the right external jugular vein into a fraction collector with sampling times ranging from 0.25 to 4 s over a 28-min sampling period. A total of 62 concentration data points were used in the pharmacokinetic modelling of each impulse response curve data set.

Assay

A 20-μL sample from each outflow sample containing the [^{99m}Tc]RBC and [^{3}H]- or [^{14}C]-solute was transferred to Eppendorf tubes for counting in a Cobra II Auto-Gamma counter (Packard Instrument Co., US). After allowing the technetium in the samples to decay for at least three days, a! further sample from each outflow sample (20 μL) was transferred to scintillation vials containing 5 mL Ultima-Gold scintillation fluid (Packard Instrument Co., USA). The samples were then counted in a Minaxi β Tri-Carb 4000 series liquid scintillation counter (Packard, USA). The number of counts obtained from both β- and γ-samples were compared with the counts of the standards prepared from the injectate. Concentrations of the ^{14}C/^{3}H-solute or [^{3}H]water and [^{99m}Tc]RBC in each sample were expressed as the fraction of the dose injection per millilitre of perfusate.

Viability parameter determination

During each experiment, the viability of the brain/head preparation was assessed by a number of criteria, namely cerebral oxygen consumption, pH, enzyme release and perfusion pressure (Foster et al 2000). During the bolus studies, perfusion pressure was stable at approximately 70–80 mmHg.

Fraction unbound in perfusate

The unbound fraction (f_{up}) of a drug was determined by ultrafiltration using a micropartition system (Amicon Separations Centricon centrifugal filter devices (YM-30), Millipore Corporation, Bedford, MA). Appropriate validation was performed according to Wu (1996). The radiolabelled drug ($\sim$ 1.0 μCi mL^{-1} [^{14}C]diclofenac, [^{3}H] clonidine or [^{14}C]salicylate) was dissolved in MOPS buffer (pH 7.4; with 2% BSA) and incubated in a water bath at 37°C for at least 30 min. Before centrifugation, 100-μL samples of the drug/buffer solution were placed into scintillation vials containing 2 mL scintillation fluid and counted for radioactivity. Samples (1 mL) of the drug/buffer solution were pipetted into the Centricon centrifugal filter devices and centrifuged at 7000 rev min^{-1} ($\sim$ 3840 g) for 20 min at 29°C using a Beckman J2-21 centrifuge with JA-20 rotor (Beckman, Palo Alto, CA). Samples (100 μL) were then taken from the filtrate in the bottom of the filter device, added to scintillation vials containing 2 mL scintillation fluid and counted for radioactivity. The f_{up} of the drug was calculated as the ratio of the radioactivity of the drug in the filtrate and the radioactivity of the drug in the buffer before centrifugation.

Assay for radioactivity in brain tissue

At the end of a perfusion, the brain was removed and divided into the regions of left cortex, right cortex, cerebellum and medulla, and subcortex. Each weighed section was placed into scintillation vials with 5 M sodium hydroxide and allowed to digest for two to three days at 70°C. Scintillation fluid (4 mL) was then added to the vials and the tissues were counted for radioactivity.

Solute lipophilicity

The logarithm of the octanol–water partition coeffi cients was based on predictive values using the program clog P 1.0.0 for Macintosh, Biobyte Corporation, Claremont, CA, 1994.

Pharmacokinetic model

The outflow concentration–time curve $C_{IR}(t)$ after bolus administration of dose (D) into the isolated head with flow rate (Q) (i.e. the impulse response function) of the intra-vascular marker [^{99m}Tc]RBC and [^{3}H]water was 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 (transit time density functions

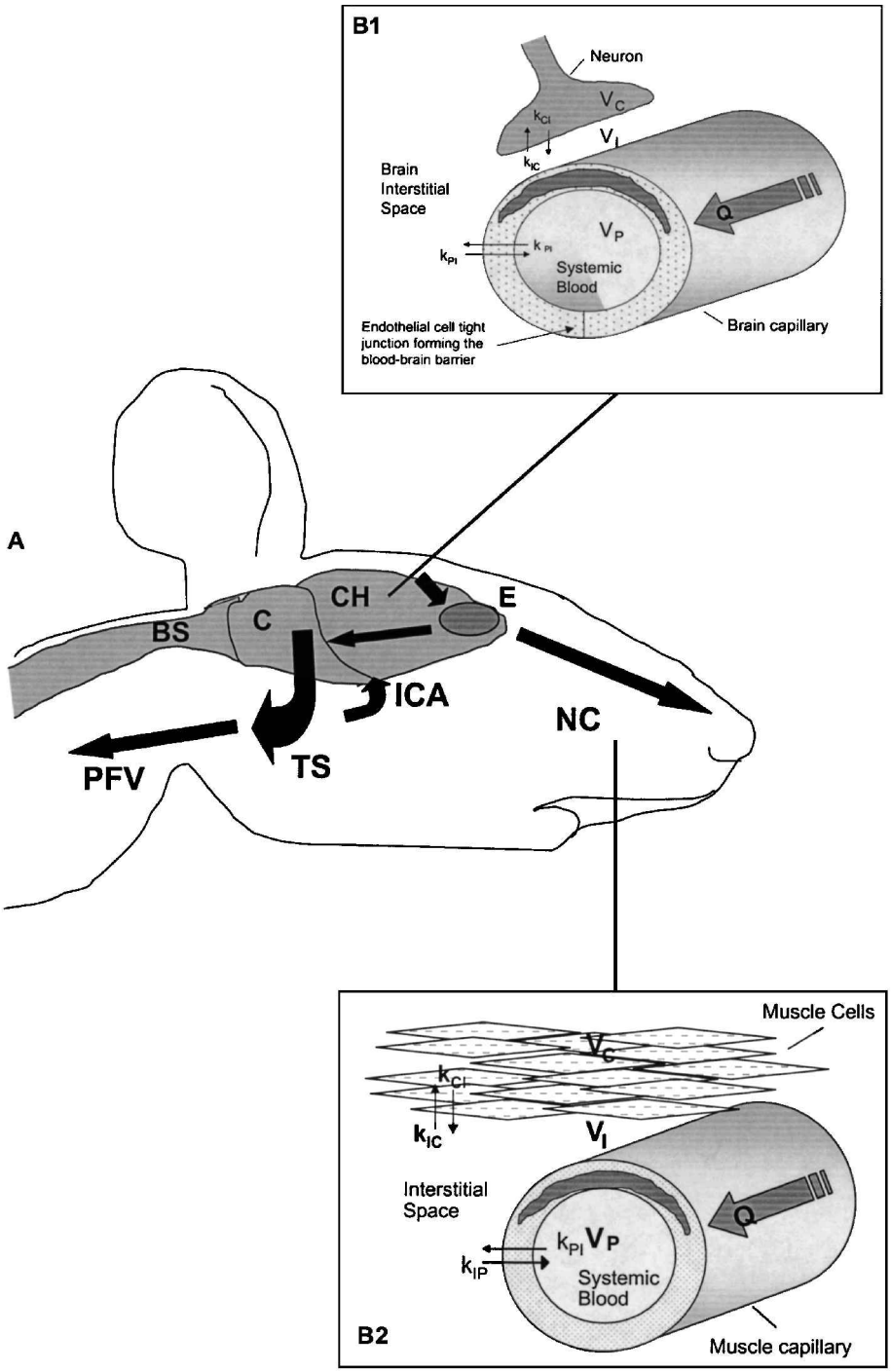


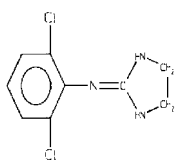
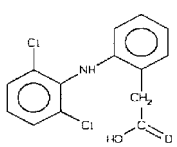
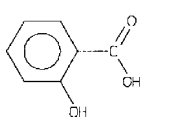
Figure 1 The path of the perfusate in the isolated in-situ perfused rat head and model of two permeability barriers in the head. A. The perfusate is depicted as entering the brain via the right internal carotid artery (ICA). As described by Foster et al (2000), the perfusate is able to exit the brain via the superior sagittal sinus or the arteries, which supply the eye (E). In the latter case, the perfusate is thought to be able to enter the nasal cavities (NC) and surrounding muscle before being emptied into the transverse sinus (TS) followed by the posterior facial vein (PFV). Abbreviations for the brain regions depicted in the diagram include the brain stem (BS), cerebellum (C) and cerebral hemisphere (CH). B. Model of two permeability barriers in (1) brain and (2) muscle used to describe the kinetics of a drug in the isolated perfused rat head preparation. The flow rate is represented by Q.

Table 1 Viability parameters obtained from the in-situ perfusion experiments[a].

Solute examined	Oxygen consumption (μmol min^{-1} g^{-1})	Creatine kinase release (U L^{-1})	Lactate dehydrogenase release (U L^{-1})	pH % change
Clonidine	2.33±0.16	0±0	6.33±4.80	2.14±0.33
Diclofenac	2.46±0.11	19.5±8.72	32.33±23.35	2.36±0.44
Salicylate	2.26±0.11	5.17±3.06	7.33±3.33	2.06±0.23

Values are presented as mean±s.d., n = 3. [a]Each viability parameter indicates levels in the outflowing perfusate, except for pH where the percentage change between inflowing and outflowing pH is given.

Table 2 Physicochemical properties of solutes studied (in alphabetical order with mean±s.d., where applicable).

Solute	Chemical structure[a]	pK$_a$[b]	Fraction un-ionized at pH 7.4[c]	Molecular weight (Da)[d]	clog P[a]	f$_{up}$
Clonidine		8.25 (B)	0.1237	230.10	1.367	0.84±0.006
Diclofenac		4.00 (A)	3.98e−5	318.13	3.031	0.0095±0.00005
Salicylate		2.97 (A)	3.72e−5	138.12	2.187	0.17±0.002
Sucrose		12.62 (A)	6.02e−6	342.30	−5.718	1.0[e]

[a]Calculated using clog P 1.0.0 for Macintosh, Biobyte Corporation, Claremont, CA, 1994. [b]Speight & Holford (1997). [c]Calculated by the equations, for acids: [100/{1+antilog(pH−pK$_a$)}]/100; for bases: [100/{1+antilog(pK$_a$−pH)}]/100. [d]Budavari (1996). [e]Simple sugars have an f$_{up}$ of 1.0 (Wu et al 1995).

(TTD) are denoted by f(t)). The $C_{IR}(t)$ data of permeating compounds were analysed using an extended version of the two-phase organ model (Weiss & Roberts 1996). The model assumes drug transport in a random capillary network with permeation across the endothelial barrier and non-instantaneous equilibration in the tissue phase. The latter is governed by instantaneous distribution in the interstitial and intracellular space and permeation across the cell membrane (refer to Figure 1). The model equations have been fully described by Hung et al (2001). The data were fitted using the non-linear regression program SCIENTIST (MicroMath Scientific Software, Salt Lake City, UT).

Statistical analysis

All values are expressed as mean±s.d. Results were analysed using analysis of variance and Tukey with

GraphPad Prism, version 3.02 (GraphPad Software, San Diego, CA). $P < 0.05$ was accepted as the value of statistical significance.

Results

Table 1 shows the viability parameters for the experiments conducted. The level of oxygen consumption was consistent for all experiments. In each experiment, creatine kinase, which is a sensitive indicator of brain damage, was minimally released. Lactate dehydrogenase, a less sensitive indicator of damage in the brain compared with creatine kinase, also exhibited low levels in each experiment. The pH values for the outflowing perfusate for each experiment were reduced by 0.1 to 0.2 U relative to the inflowing pH values. This was expected since oxygen in the perfusate was utilized by the brain and carbon dioxide was released into the venous perfusate as a result. Carbonic acid was produced from the presence of the dissolved carbon dioxide resulting in a reduction of pH compared with the inflowing perfusate. Overall, this was a viable preparation due to the consistent levels of oxygen and low levels of enzymes released during each experiment.

The solutes chosen in this study differed widely in their physicochemical properties (Table 2). The fraction of solute unbound in perfusate (f_{up}) ranged from 1 (water and sucrose) to 0.0095 (diclofenac) (Table 2). The logarithm of the octanol–water partition coefficient also varied widely from −5.718 for sucrose to 3.031 for diclofenac.

Figure 2a shows a representative fit of the concentration–time profiles of the vascular marker [^{99m}Tc]RBC using the double inverse Gaussian function. Figure 2b–f shows respective fits for the solutes [^{14}C]diclofenac, [^{3}H]clonidine, [^{14}C]salicylate, [^{14}C]sucrose and [^{3}H]water using the physiological two-barrier model (also referred to as a stochastic three-phase model). In each case, the two models used were able to aptly describe the outflow data.

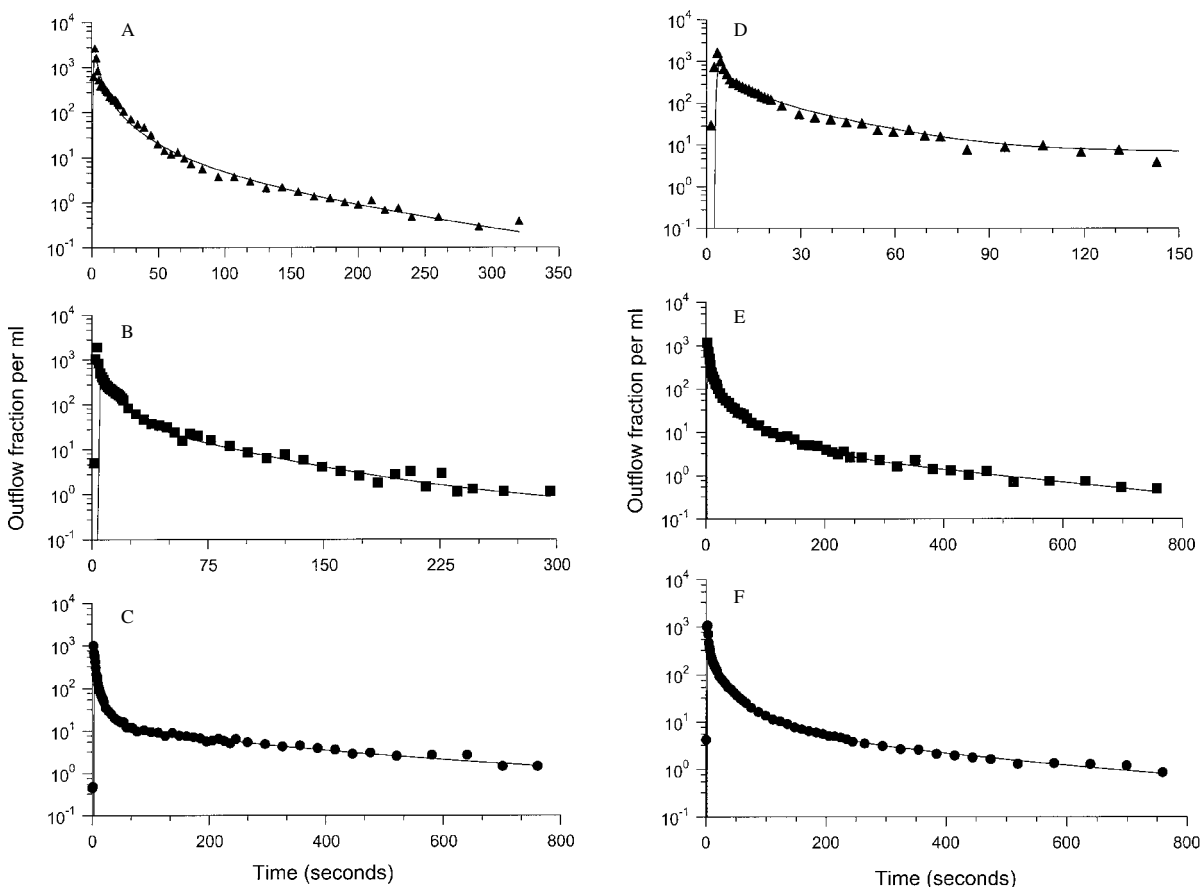


Figure 2 Outflow concentration–time data fits of [^{99m}Tc]RBC (A) using the double inverse Gaussian function and of the solutes [^{14}C]diclofenac (B), [^{3}H]clonidine (C), [^{14}C]salicylate (D), [^{14}C]sucrose (E) and [^{3}H]water (F) using the two-barrier model.

Table 3 Distribution volumes, clearance values and PS products of various solutes in the isolated perfused rat head at 37°C and with 2% BSA in the perfusate obtained by two-barrier model analysis of impulse response data.

Parameter	Clonidine	Diclofenac	Salicylate	Sucrose	Water
Vd_P (mL)	1.07±0.18	1.03±0.24	1.58±0.25	1.15±0.36	1.15±0.24
Vd_I (mL	2.78±0.47	2.02±0.46	2.44±1.70	3.12±1.80	4.24±0.60
Vd_C (mL)	14.30±6.17	20.90±8.04	3.91±2.70	20.16±16.81	8.23±0.82
k_{IC} (s^{-1})	0.05±0.02	0.02±0.01	0.03±0.02	0.019±0.015	0.005±0.001
k_{CI} (s^{-1})	0.010±0.004	0.002±0.001	0.05±0.03	0.010±0.014	0.006±0.004
CL_{PI} (mL s^{-1})	0.41±0.16	0.40±0.17	0.39±0.34	0.36±0.11	0.59±0.11
CL_{IC} (mL s^{-1})	0.14±0.07	0.04±0.02	0.09±0.11	0.400±0.003	0.02±0.01
K_P[a]	1.37	1.84	0.51	1.87	
PS_{PI} (mL s^{-1})[b]	0.48±0.19	41.79±17.96	2.32±1.98	0.36±0.11	

Values are presented as mean±s.d., n = 3, but for water n = 8. [a]Fractional distribution volumes (K_P) are derived from mean data: n = 3 for each drug and n = 8 for water. [b]PS_{PI} values calculated from CL_{PI} and f_{up} (as shown in Table 2). CL_{PI}, plasma to interstitial fluid permeation clearance. k_{CI}, transfer rate constant between cellular and interstitial distribution volumes. k_{IC}, transfer rate constant between interstitial and cellular distribution volumes. PS_{PI}, permeability–surface area product. Vd_I, interstitial volume. Vd_C, intracellular volume. Vd_P, vascular volume. f_{up}, fraction unbound in perfusate.

Table 4 Comparison of physicochemical and pharmacokinetic factors of the solutes used in this study.

Solute	PS_{PI}	f_{up}[b]	f_{ut}	clog P
Clonidine	0.48±0.19	0.84±0.006	0.61	1.367
Diclofenac	41.79±17.96[a]	0.0095±0.00005	0.0052	3.031
Salicylate	2.32±1.98	0.17±0.002	0.33	2.187
Sucrose	0.36±0.11	1.00	0.53	−5.718

Where applicable, values are represented as mean±s.d., n = 3. [a]$P < 0.01$ compared with the PS_{PI} values for the other solutes. [b]$P < 0.001$ compared with the f_{up} for the other solutes. f_{up}, fraction unbound in perfusate. f_{ut}, fraction unbound in tissue. PS_{PI}, permeability–surface area product.

Table 3 shows the pharmacokinetic parameters estimated for the solutes studied and for water using the outflow concentration–time profiles after bolus injection and regression with a physiological two-barrier model. The vascular distribution volume (Vd_P) was similar for all solutes and was, on average, 1.19 A similar interstitial distribution volume (Vd_I) of 2.59 mL was seen for all solutes other than water, where the Vd_I was 4.24 mL (Table 3). In contrast, marked variations in the cellular distribution volumes (Vd_C) for the solutes were found. Diclofenac and sucrose had the highest values followed by clonidine and salicylate. Similarly, the fractional distribution volume K_P was largest for sucrose and diclofenac followed by clonidine and salicylate. The fractional distribution volume obtained for each drug indicated the extent of binding in the interstitial and cellular spaces. Values of less than 1.0 represented the exclusion of a drug from the space. A value equal to 1.0 indicated no binding and a value of more than 1.0 represented binding of a drug within the space. Diclofenac, clonidine and sucrose had values of more than 1.0 as compared with salicylate, which had a value of less than 1.0.

Table 4 shows the estimated endothelial permeability–surface area (PS) products and average fraction unbound in the tissues, together with clog P and f_{up} from Table 2. It was apparent that those solutes that were more lipophilic had a higher PS product with the exception of sucrose. For example, the PS product of diclofenac was significantly greater ($P < 0.01$) than the other solutes examined. In addition, the f_{up} values for each solute differed significantly ($P < 0.001$) from the other solutes investigated.

Table 5 shows the radioactivity remaining in different parts of the brain 28 min after bolus injection for the solutes used in this study. It was apparent that, as a fraction of the dose administered, the radioactivity levels were very low, indicating that most of the solutes had been removed from the head at the time of analysis. The levels appeared, within error, relatively similar for the different parts of the brain.

Discussion

The transport of drugs across the various barriers of the head is an important determinant in the effective treat-

Table 5 Radioactivity ([^{3}H] and [^{14}C]) remaining in different regions of the rat brain following in-situ head perfusion experiments (approximately 28 min after the injection) conducted at 37°C, 2% BSA and at 4.6 mL min^{-1}.

Solute	Estimated remaining fraction of dose injected/g brain			
	Left cortex	Right cortex	Cerebellum and medulla	Subcortex
Clonidine	0.0210±0.002	0.058±0.016	0.028±0.018	0.035±0.002
Dicolfenac	0.0020±0.0005	0.002±0.001	0.0050±0.0006	0.0035±0.0004
Salicylate	0.070±0.072	0.069±0.075	0.085±0.052	0.059±0.046
Sucrose	0.00051±0.00044	0.00071±0.00047	0.00038±0.00017[b]	0.00067

Values are presented as mean±s.d., n = 3. [a]Remaining radioactivity for sucrose is based on n = 2 except for the subcortex which is n = 1. [b]Calculation is based on cerebellum only.

ment of disorders of the brain and other areas of the head. Most studies to date have emphasized transport in the brain as a single organ (Bonate 1995). This study examined pharmacokinetics in the head. The head, in context to the brain, is a heterogeneous region consisting of a number of organs of which the brain is only one. However, the pharmacokinetic modelling and analysis of solute transport in the head is scarce in the literature. The purpose of this study was threefold. Firstly, we sought to examine the disposition of solutes with different physicochemical properties in an isolated in-situ rat head perfusion model using a sampling time of 28 min, which would allow adequate recovery of the drug in the perfusate. Secondly, we sought to model the disposition of the solutes in the head using a physiologically based pharmacokinetic model. Thirdly, we sought to interrelate the pharmacokinetic parameters derived from the model with the physicochemical properties of the solutes.

RBC were used in this preparation as the vascular marker for the perfused head. In each case, they exhibited a 100% recovery calculated from moments analysis (Foster et al 2000). This result as well as the short mean transit times as compared with the solutes studied, indicated that the RBC remained in the vascular space, did not penetrate into the interstitial or cellular spaces and were fully recovered in the venous outflow.

Whereas a double-inverse Gaussian function was adequate to describe the kinetics of RBC in the perfused head, a physiological two-barrier model was required to fit the outflow concentration–time data for the drugs and other solutes. Salicylate, clonidine and diclofenac were more lipid soluble and therefore were able to cross vascular (e.g. blood–brain barrier) and cellular barriers in the head to varying extents to exert their pharmacological effects. Therefore, the permeability or permeation clearance for a solute diffusing from the perfusate into the interstitial space and finally the cellular space was required.

The distribution volumes of RBC in the perfused head, determined in the different experiments, in the vascular or perfusate space were relatively equal. The distribution volumes of the drugs indicated that although all three drugs had relatively the same volumes for the vascular as well as interstitial spaces, all three differed in the cellular space. The small value for salicylate in the cellular volume and the larger value for debinding as compared with binding indicated that the majority of the drug was confined to the vascular and interstitial spaces of the head and was largely recovered in the venous outflow after a relatively short amount of time.

In contrast, diclofenac and clonidine both had larger cellular volumes and larger values for binding compared with debinding, indicating that these drugs remained in the cellular space for a longer period of time and were much slower to be recovered during the allotted time for venous outflow collection. The fractional distribution volumes also indicated this in that both clonidine and diclofenac had values of more than 1.0 as compared with salicylate (Table 3). Extensive clonidine uptake has been reported also in the in-vivo brain where the brain/blood concentration ratio (C_{brain}/C_{blood}) was 1.30±0.16 (Young et al 1988).

Few studies have examined the physicochemical factors for solute uptake into the perfused head. However, uptake into the whole heterogeneous system of the head appears to be decided by the same determinants that have been well described for the brain. Two of the key determinants of steady state solute uptake into the brain are lipophilicity and protein binding (Ramsay et al 1979; Tedeschi et al 1983; Hariton et al 1985; Jones et al 1988;

Lin & Lin 1990). The model used in this work recognizes the contribution of protein in the transient distribution process where the distribution volume is determined from the PS product of the capillary wall and f_{up}. The slow efflux of diclofenac (Table 3) may be related to its low average unbound fraction in the cells (Table 4), which may in turn be the result of the drug binding with albumin in the cellular space. In this case, the clearance of diclofenac would be dependent on albumin efflux. Owen et al (1994) examined the disappearance kinetics of diclofenac from synovial fluid following intra-articular administration, and they found that the binding of diclofenac with albumin in synovial fluid reduced the rate at which diclofenac returned to the blood. It was suggested that almost half of the clearance of diclofenac was in the bound form. This is in contrast to clonidine and salicylate whose efflux rates and unbound fractions in the cells were higher. Diclofenac was also more lipophilic (Table 4) than clonidine or salicylate, which may have contributed to its higher PS product than the other two drugs.

Several studies have collected outflow fractions over short periods of time and, as a consequence, underestimated the distribution volumes of solutes in their preparations. Significantly, as shown in our earlier work (Foster et al 2000), these volumes often appear to be similar to the brain. Longer sampling times show that the flow dispersion (determining the transit time dispersion of solutes) is indeed much greater in the head as is evidenced through the vascular casting and fluoroscopic imaging of the perfusion (Foster et al 2000). The study conducted by Sakane et al (1991) was the first to use moments analysis to describe the influx and efflux of vascular markers and various drugs in the head. Similar to our perfusion model, the outflowing perfusate was collected from the posterior facial vein and not from a vein directly collecting perfusate from the brain such as the superior sagittal sinus. This meant that areas in the head other than the brain were also perfused in this preparation (refer to Foster et al (2000) and Figure 1). However, the collection time was limited to 40 s, which resulted in an underestimate of the tail of the outflow curve and of the distribution volume.

An incomplete determination of the tail component may be a major limitation in past brain studies as well. For instance, Kassissia et al (1995) examined the pharmacokinetics of a number of solutes including labelled albumin as well as water and oxygen in the in-vivo dog brain. In that case, the venous samples were taken directly from the dorsal sagittal sinus ensuring that the brain was the organ being studied. However, the sampling time was limited to 25 s and therefore was insufficient to allow the total solute efflux from the brain to be quantified. This would have resulted also in underestimations of the pharmacokinetic parameters measured.

Conclusions

A viable in-situ perfusion technique along with a two-barrier model were successfully used to determine the pharmacokinetics of a range of solutes with varying physicochemical properties in the head. This study has important implications in terms of the effective treatment of disorders of the brain and other areas of the head.

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