The Effect of Blood Sampling Site and Physicochemical Characteristics of Drugs on Bioavailability after Nasal Administration in the Sheep Model

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Purpose. Investigate the effect of blood sampling site and physicochemical characteristics of drugs on the pharmacokinetic (PK) parameters obtained after intravenous and nasal administration in sheep and compare results with computer simulations.

Methods. Three drugs, insulin, morphine, and nicotine, were administered nasally and by intravenous (IV) injection to sheep, and serial blood samples collected concurrently from the carotid artery (insulin, morphine) or cephalic vein (nicotine) and jugular vein. Plasma drug concentrations were measured, and pharmacokinetic and statistical analyses performed, to evaluate sampling site differences.

Results. After nasal insulin, bioavailabilities calculated from the two blood sampling site data were comparable. In contrast, apparent bioavailabilities following nasal morphine or nicotine were significantly higher when sampling was from the jugular vein. These results were supported by computer simulations. These observations are attributed to the greater effects of noninstantaneous mixing of drugs for jugular vein sampling following nasal dosing, compared to the other sampling sites, which is significant for drugs that are rapidly and well absorbed and that have a high volume of distribution (V_d).

Conclusion. The results clearly show that the characteristics of the drug and the blood sampling site can have a significant effect on the pharmacokinetic results obtained after nasal administration in sheep.

KEY WORDS: sheep; insulin; morphine; nicotine; nasal administration; blood sampling.

INTRODUCTION

In recent years, the nasal route has been increasingly evaluated for delivery of drugs used in acute therapeutic treatments, for example, in breakthrough pain, erectile dysfunction, nausea, and migraine, where a rapid onset of action is essential for an optimal effect. Furthermore, the nasal route has also been evaluated for delivery of challenging drugs such as peptides and proteins that are not easily administered by nonparenteral routes of delivery and that are often used in long-term therapies such as in treatment of bone diseases, short stature, and diabetes. Several nasal products in these categories are now marketed and include the antimigraine drugs sumatriptan (GlaxoSmithKline) and zolmatriptan (AstraZeneca) as well as calcitonin (Novartis) for the treatment of osteoporosis (1).

Despite the relatively large surface area (created by the three turbinate structures) and the well vascularized epithelium of the nasal cavity, small polar drugs and large hydrophilic molecules are not readily absorbed across the nasal membrane. Bioavailabilities are normally less than 10% for small polar drugs and 1% for peptides. Hence, drug products often need to be carefully formulated, either by using absorption promoters or other drug delivery concepts such as bioadhesive systems (2).

The development of a nasal formulation for such drugs will often require extensive preclinical studies to facilitate the selection of one or more candidate formulations for progression to human clinical trials. Traditionally, early-phase pharmacokinetic nasal studies have been performed using a surgically modified rat model, which has excellent screening potential (3). However, because of the anesthetics used in these animals, the nasal mucociliary clearance mechanism is normally partially impaired, and thus, the bioavailabilities obtained are most often significantly overestimated compared to those obtained in man (4,5). Furthermore, dry powder nasal formulations cannot easily be tested in this model. Certain groups in the United States, Japan, and Europe have used conscious or lightly sedated dogs in nasal pharmacokinetic studies and found that this model can give results comparable to those obtained in man. However, for legislative, ethical, and economic reasons, this species is becoming increasingly difficult to use for preclinical experimentation unless for regulatory (toxicologic) studies.

In line with some other research groups, for a number of years we have employed the sheep as a preclinical model for the development of nasal formulations (6–8). The sheep is a less emotive animal than the dog and is farmed for consumption. It is docile and easy to handle during experimental procedures and can be used in crossover design experiments. The nasal cavity of the sheep has a larger surface area than that of man, but on a surface area per kilogram body weight basis (8.2 cm^2/kg) the value is comparable to that of man (2.5 cm^2/kg). For comparison, values for other species are monkey 7.7, dog 22.0, and rat 41.6 cm^2/kg. In addition, in the sheep, the percentage of the nasal area that constitutes the olfactory region is comparable to that in man.

For convenience, it has been normal practice to sample blood from the jugular vein of the sheep during nasal drug delivery studies. However, venous blood from the nasal cavity drains into the jugular veins before entering the general circulation. As a consequence, the measured drug concentration following jugular vein sampling after nasal dosing could be overestimated and in turn could also affect calculated bioavailability values. The purpose of the present investigation was to compare results from three studies performed in the sheep model using three different drugs and to investigate the effect of the blood sampling site and characteristics of drugs on the resultant measured pharmacokinetics obtained after nasal delivery. The sampling sites chosen in the different studies were the jugular vein, draining blood from the head to the right part of the heart, the carotid artery supplying blood from the left part of the heart to the head, or the cephalic vein, which drains blood from the forelimbs (in humans, the arms) via the axillary veins and the external jugular vein to the right part of the heart. The drugs selected were the peptide insulin,

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the low-molecular-weight polar drug morphine, and the more lipophilic drug nicotine. Computer simulations were carried out in order to predict the key factors affecting the pharmacokinetic profiles of the drugs sampled by the alternative routes.

MATERIALS AND METHODS

Materials

Morphine hydrochloride trihydrate was obtained from MacFarlan Smith Ltd. (Edinburgh, UK). Human recombinant sodium insulin and nicotine hydrogen tartrate (2.5 H_2O) were obtained from Sigma (Poole, UK). The characteristics of the various drugs are given in Table I. Chitosan glutamate (Seacure G 210) was purchased from Pronova Biomedical (Oslo, Norway). Amberlite IRP69 ion-exchange resin was purchased from Aldrich Chemical Company (Milwaukee, WI). Sterile saline solution was from Fresenius Healthcare Group (Basingstoke, UK). All other materials were of pharmaceutical or analytic grade.

Preparation of Formulations

Insulin Formulations

A sterile isotonic solution formulation of sodium insulin (2 IU/ml) was prepared for intravenous dosing (F1), and a sterile insulin-chitosan solution formulation comprising 200 IU/ml insulin and 5 mg/ml chitosan glutamate was prepared for nasal dosing (F2).

A powder formulation containing 7.7% w/w sodium insulin and chitosan glutamate powder was prepared by dry-blending for nasal dosing (F3). The formulation contained approximately 1.8 IU insulin per milligram powder formulation. The insulin content in the intravenous and nasal formulations was confirmed by HPLC. A summary of the formulations is given in Table II.

Morphine Formulations

A sterile isotonic solution formulation of morphine hydrochloride trihydrate (1.32 mg/ml equal to 1.0 mg/ml anhydrous morphine base) was prepared for intravenous dosing (F4). A dry-blend powder formulation containing morphine hydrochloride trihydrate (the equivalent of 12.5% w/w anhydrous morphine base) and chitosan was prepared for nasal dosing (F5). The morphine content in the intravenous and nasal formulations was confirmed by HPLC. A summary of the formulations is given in Table II.

Table I. Characteristics of Drugs Employed in the Studies

	MW	Log *P* (octanol/water)	V_d(h) ($L \cdot kg^{-1}$)	Protein binding (%)
Morphine	303.4	0.89	2.6–4.0	25–35
Nicotine	162.2	1.17	2.6	5
Insulin	5300	–1.08*	0.07–0.14	5

* Butanol/water.
V_d(h) = Volume of distribution in humans.
MW = molecular weight in daltons.

Nicotine Formulations

A sterile isotonic solution of nicotine hydrogen tartrate (0.78 mg/ml, equivalent to 0.25 mg/ml nicotine base) (F6) and a solution of nicotine hydrogen tartrate (15.7 mg/ml, equivalent to 5 mg/ml nicotine base) (F7) were prepared for intravenous and nasal administration, respectively.

A lyophilized powder was prepared from nicotine hydrogen tartrate and Amberlite IRP69. The powder contained 1.06 mg drug/mg resin or the equivalent of 18% w/w nicotine base (0.18 mg nicotine per 1 mg powder) as previously described (9) (F8). The content of nicotine in the formulations was confirmed by UV spectroscopy.

In Vitro Analytic Assays

Insulin

The insulin concentrations of the various formulations were analyzed using a Gilson HPLC system fitted with a Vydac C_{18} 5-μm pre-column and a Vydac reverse-phase C_{18} 5-μm 150 × 4.6 mm column (Hichrom, Reading, UK). Gradient conditions and a flow rate of 1.0 ml/min (ambient temperature) were used. The mobile phase was composed of eluent A, containing 95% ethanolamine (0.6%, pH3) and 5% acetonitrile, and eluent B, containing 40% ethanolamine (0.6%, pH3) and 60% acetonitrile. The injection volume was 50 μl. The ultraviolet detector was set at 210 nm. The analysis run time was 18 min. Samples were prepared for analysis by dissolving/diluting the formulation in 0.08 M sodium dodecyl sulfate/0.6% ethanolamine (pH3):methanol (99:1).

Morphine

The morphine hydrochloride analysis was performed by reverse-phase HPLC with ultraviolet detection by using a method slightly modified from the assay method described by Svensson *et al.* (10). The limit of detection of the assay was 10 μg/ml.

For the liquid formulation, the samples were diluted 1000 times in the HPLC mobile phase and analyzed in duplicate. For the powder formulation, 30-mg samples were dispersed in 26 ml of acetonitrile and made up to 100 ml with HPLC buffer. Each sample was filtered through 0.8-μm filters (Sartorius) and analyzed in duplicate by HPLC. The calibration curve used for all samples covered the concentration range 10 to 100 μg/ml.

Nicotine

The concentration of nicotine in the various formulations was analyzed at 260 nm using a Hewlett Packard 8452A Diode Array Spectrophotometer as described previously (9).

Sheep Studies

Separate groups of crossbred (Suffolk and Texel) sheep were used in each study. The sheep were housed indoors for the duration of each trial and were fed hay supplemented with a pelleted concentrate and water *ad libitum.* Before the start of the insulin and morphine dosing studies, the carotid artery and the external jugular vein of each sheep were cannulated under general anesthesia (intravenous thiopentone sodium/inhalation of fluothane, nitrous oxide, and oxygen). The

Table II. Formulations Administered to Sheep

Formulations	Morphine[a] (mg/kg)	Insulin (IU/kg)	Nicotine[b] mg/kg	Chitosan (mg/kg)	Dose volume (ml/kg)/(mg/kg)[c]	Drug content[a,b]
F1	–	0.1	–	–	0.05	2 IU/ml
F2	–	2.0	–	0.05	0.01	200 IU/ml
F3	–	2.0	–	1.0	1.03[c]	1.8 IU/mg
F4	0.1	–	–	–	0.1	1.0 mg/ml
F5	0.3	–	–	2.0	2.4[c]	12.5% w/w
F6	–	–	0.04	–	0.16	0.25 mg/ml
F7	–	–	0.04	–	0.01	5 mg/ml
F8	–	–	0.1	–	0.55[c]	0.18 mg/mg

[a] Anhydrous morphine base.
[b] Nicotine base equivalent.
[c] Powder.

sheep were allowed to recover from the effects of the surgery before being used in the pharmacokinetic studies. In the nicotine dosing study, blood samples were collected by venipuncture of the cephalic veins under local anesthesia [EMLA cream (Astra Pharmaceuticals, Kings Landley, UK) applied topically]. A jugular vein was also cannulated under local anesthesia (subcutaneous infiltration of lidocaine) using a Seldinger technique. The patency of all vascular cannulas was maintained by flushing them through with sterile heparinized (25 IU/ml) normal saline.

To facilitate nasal dosing (for restraint and as a countermeasure against the animals sneezing and snorting during drug administration) the sheep were sedated (for about 3 min) with a dose of 2.25 mg/kg ketamine hydrochloride (Vetalar®, Fort Dodge Animal Health Ltd., Southhampton, UK) administered via a jugular vein. For consistency, ketamine was also administered before intravenous dosing of the chosen drug. There was a minimum washout period of 2 to 5 days between successive dosings in each sheep depending on the drug administered. During the studies, the maximum volume of blood taken from each animal was less than 15% of the total blood volume. Hence, the blood sampling is expected to have had minimal physiologic effect on the animals. The sheep studies adhered to the "Principles of Laboratory Animal Care" (NIH publication number 85-23, revised 1985) and were performed under a valid Home Office (United Kingdom Government) Project Licence and had received approval by the Ethical Review Committee at the University of Nottingham.

Administration of Drugs to Sheep

Insulin

Administration of Insulin Formulations. The study was performed as a crossover study in a group of three sheep weighing 63 to 67 kg. The intravenous insulin solution (F1) was administered at 0.05 ml/kg (0.1 IU/kg) as a bolus by direct venipuncture of a jugular vein (contralateral to the cannulated jugular vein) using an infusion set. The residual dose in the infusion set was flushed into the vein with 2 ml of 0.9% sterile normal saline.

The nasal insulin solution formulation (F2) was administered at 0.01 ml/kg using a syringe and a Blueline umbilical cannula (Portex, UK) inserted approximately 7 cm into each naris. The nasal insulin powder formulation (F3) was administered into either one or both nares in separate legs of the study, using Blueline siliconized oral/nasal tracheal tubes containing the preweighed dose, inserted approximately 7 cm into the nares and delivered by means of a simple one-way spray bellows.

Plasma Sampling. Blood samples of 3 ml were collected concurrently from the carotid artery and the jugular vein via the respective cannulas for up to 300 min after dosing at appropriate intervals. Before collection of each sample, the initial 1.5 ml of blood collected was discarded to eliminate traces of heparinized saline.

The blood samples were mixed gently in heparinized tubes (4 ml, containing 60 IU lithium heparin) (Sarstedt, Leicester, UK) and stored on crushed ice before separation of plasma. Plasma was separated within 15 min of sample collection by centrifugation at room temperature at approximately 3000 rpm (1600 g). The plasma was stored in polystyrene tubes at –80°C awaiting analysis of insulin content.

Plasma Analysis. The plasma insulin levels were determined by radioimmunoassay using a double-antibody technique developed in the City Hospital (Nottingham, UK) as described previously (11). This assay is for human insulin (using an antibody raised in guinea pigs), with a second antibody (an anti–guinea pig antibody) used to precipitate the insulin–insulin antibody complex. In preliminary experiments it was established that the addition of human insulin to sheep plasma yielded 80 to 85% recovery of the added material on assay.

Morphine

Administration of Morphine Formulations. The study was performed as a crossover study in a group of three sheep weighing 55 to 59 kg. The intravenous morphine solution (F4) was administered at 0.1 ml/kg as a bolus by direct venipuncture of the jugular vein (contralateral to the cannulated jugular vein) as for insulin. The residual dose in the infusion set was flushed into the vein with 2 ml of 0.9% sterile saline.

The nasal morphine powder formulation (F5) was administered into both nares of each sheep using Blueline siliconized oral/nasal tracheal tubes as described above for insulin.

Plasma Sampling. Blood samples of 6 ml were collected concurrently from the carotid artery and the jugular vein via the respective cannulas for up to 180 min after dosing at appropriate intervals. Before collection of each sample, the

initial 3.5 ml of blood collected was discarded to eliminate traces of heparinized saline. The blood samples were dispensed into heparinized tubes (10 ml, containing 150 IU lithium heparin) (Sarstedt, Leicester, UK), mixed gently, and stored on crushed ice before separation of plasma. Plasma was separated within 15 min of sample collection by centrifugation at room temperature at approximately 3200 rpm (1800 *g*). The plasma was stored in polystyrene tubes at –20°C awaiting analysis of morphine content.

Plasma Analysis. The plasma morphine levels were measured in the sheep plasma samples by a solid-phase quantitative radioimmunoassay by using a commercial Coat-A-Count serum morphine kit (Diagnostics Product Corporation, Abingdon, Oxfordshire, UK) as described previously (12). The RIA-CALC programme was used for calculating the plasma morphine concentrations in nanomoles per milliliter by using a morphine calibration curve. Validation of the assay showed the intraday and interday variation to be within the acceptable range (CV < 15%). The limit of detection was found to be 2.8 nM. The cross-reactivity of the method with M-6-G and M-3-G metabolites was reported as negligible (12). All samples were analyzed at least in duplicate.

Nicotine

The study was performed as a crossover study in a group of five sheep weighing 45 to 50 kg. The intravenous nicotine solution (F6) was administered at 2.0 mg nicotine base equivalent per sheep as described for insulin. The nasal nicotine solution formulation (F7) was administered at the same dose via a nasal spray device inserted approximately 7 cm into the nares. The nasal nicotine powder formulation (F8) was administered at 5.0 mg nicotine base equivalent per sheep as described for insulin. The liquid and the powder nasal doses were delivered via both nares.

Plasma Sampling. Blood samples of 3 ml were collected concurrently from the cephalic vein (by venipuncture) and the jugular vein (via the sited cannula) for up to 240 min after dosing at appropriate intervals. Before collection of the jugular vein sample, the initial 1.5 ml of blood collected was discarded to eliminate traces of heparinized saline.

The blood samples were mixed gently in 10 ml heparinized tubes (Sarstedt, Leicester, UK) and stored on crushed ice before separation of plasma. Plasma was separated within 15 min of sample collection by centrifugation at 4°C and approximately 3200 rpm (1800 *g*). Plasma was collected and stored in amber glass vials at –80°C awaiting analysis of nicotine content.

Plasma Analysis. The plasma nicotine concentration was determined by a GC-MS assay as described previously (9). The assay was found to be linear over the range 0 to 150 ng/ml. The limit of detection was 0.1 ng/ml, and the limit of quantification was 0.4 ng/ml.

Pharmacokinetic and Statistical Data Analysis

Pharmacokinetic (PK) "noncompartmental" data analysis was performed using WinNonlin version 1.1 (Scientific Consulting Inc., North Carolina, USA). The principal PK parameters used to evaluate the systemic absorption of the drug and to compare the two sampling sites were the maximum blood insulin concentration (C_{max}), the time to reach C_{max} (T_{max}), area under curve (AUC), and bioavailability. Following intravenous dosing, peak plasma drug concentrations ($C_{max(extrap)}$) were also estimated for each sheep by extrapolation of the (natural) log linear line to the zero time intercept. The (absolute) bioavailability (F_{ab}) following intranasal (in) dose administration was calculated, relative to intravenous (iv) injection, according to the site of blood sampling used as follows:

$$F_{ab} = (AUC_{in} \times Dose_{iv})/(AUC_{iv} \times Dose_{in}) \times 100\%$$

For each drug, statistical analyses of the log-transformed jugular vein and corresponding carotid artery or cephalic vein PK data (except for values of T_{max}) obtained for each formulation group were compared by way of a paired *t*-test at a 95% confidence interval (interpreted as statistically significant if $p < 0.05$) using Graphpad™ InStat® version 3.01 (Graphpad Inc., San Diego, USA).

Computer Simulation

A compartmental model of the disposition of drugs following nasal administration was set up (Fig. 1). After application in the nasal cavity, the drug will be absorbed across the nasal mucosa and reach the jugular vein at a given rate ($k_{absnasal}$). From the jugular vein the drug will reach the systemic circulation ($k_{jugcirc}$) and further, at a certain rate, reach the deep compartments ($k_{circdeep}$). Elimination from the circulation will also take place ($k_{metrenal}$). From the deep compartments the drug will reappear into the circulation ($k_{deepcirc}$). The parameters used in the simulation example are given in Table III. The parameters can be changed according to the drugs modeled to give the best fit. The parameters were estimated from the available literature, in-house data, and from best estimates and included terms that consider additional factors such as clearance of the formulations from the nasal cavity to the stomach, degradation of the drug in the nasal cavity and stomach, and drug absorption from the gastrointestinal tract. The simulation was run on Mathemathica (version 2.2) (Wolfram Research Inc., Champaign, Illinois, USA) (13).

RESULTS

Insulin

The average plasma concentration–time profiles and the pharmacokinetic results after sampling of blood from the ca-

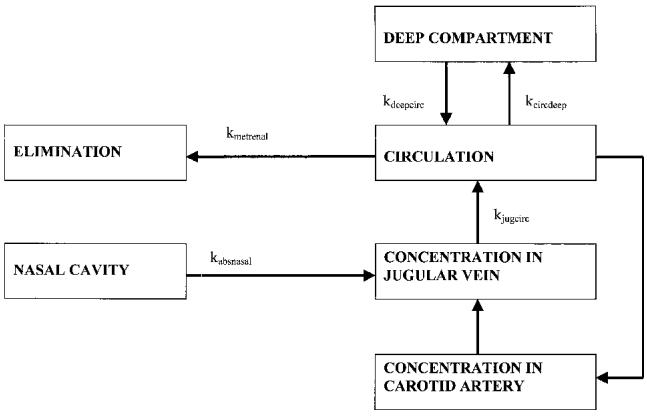

Fig. 1. Compartmental model for nasal drug distribution.

Table III. Parameters Used in the Simulation of the Drug Distribution in the Various Compartments

Dose	10 mg
Volume of distribution (V_d)	
Central	4 L
Deep	10 L
Volume of jugular compartment	200 ml
Drug half life	3.4 h
$K_{absnasal}$	0.5 h^{-1}
$K_{absstom}$	0.2 h^{-1}
$K_{clearance}$ (nose to stomach)	0.15 h^{-1}
$K_{centr/deep}$	0.5 h^{-1}
$K_{deep/centr}$	0.5 h^{-1}
$K_{jug/centr}$	25 h^{-1}
$K_{degnasal}$	0.15 h^{-1}
$K_{degstomach}$	1.5 h^{-1}
Run time	0–24 h

rotid artery and the jugular vein following intravenous and nasal administration of different insulin formulations are given in Fig. 2 and Table IV. The profiles obtained for the two sampling sites appeared similar for the different formulations. These data were also supported by the pharmacokinetic (PK) and statistical findings (Table 4); there were no significant differences ($p > 0.05$) between any PK values that were calculated from the jugular vein and carotid artery data. The resultant apparent bioavailabilities calculated for the different nasal formulations following carotid artery (CA) and jugular vein (JV) blood sampling were: 4% for the insulin–chitosan solution and 7–8% and 5–6% after dosing insulin–chitosan powder to one or two nostrils, respectively. Although the plasma profile was apparently modified with an earlier and higher C_{max} for dosing into two nostrils as compared to dosing into one nostril (Fig. 2), the pharmacokinetic values were not significantly different (Table IV). As expected from previous studies (1), the nasal insulin solution formulation gave an earlier peak plasma level of insulin as compared to the powder formulation.

Morphine

The average plasma concentration–time profiles after intravenous and nasal administration of morphine for sampling of blood from the jugular vein and the carotid artery are shown in Fig. 3. The corresponding pharmacokinetic data are

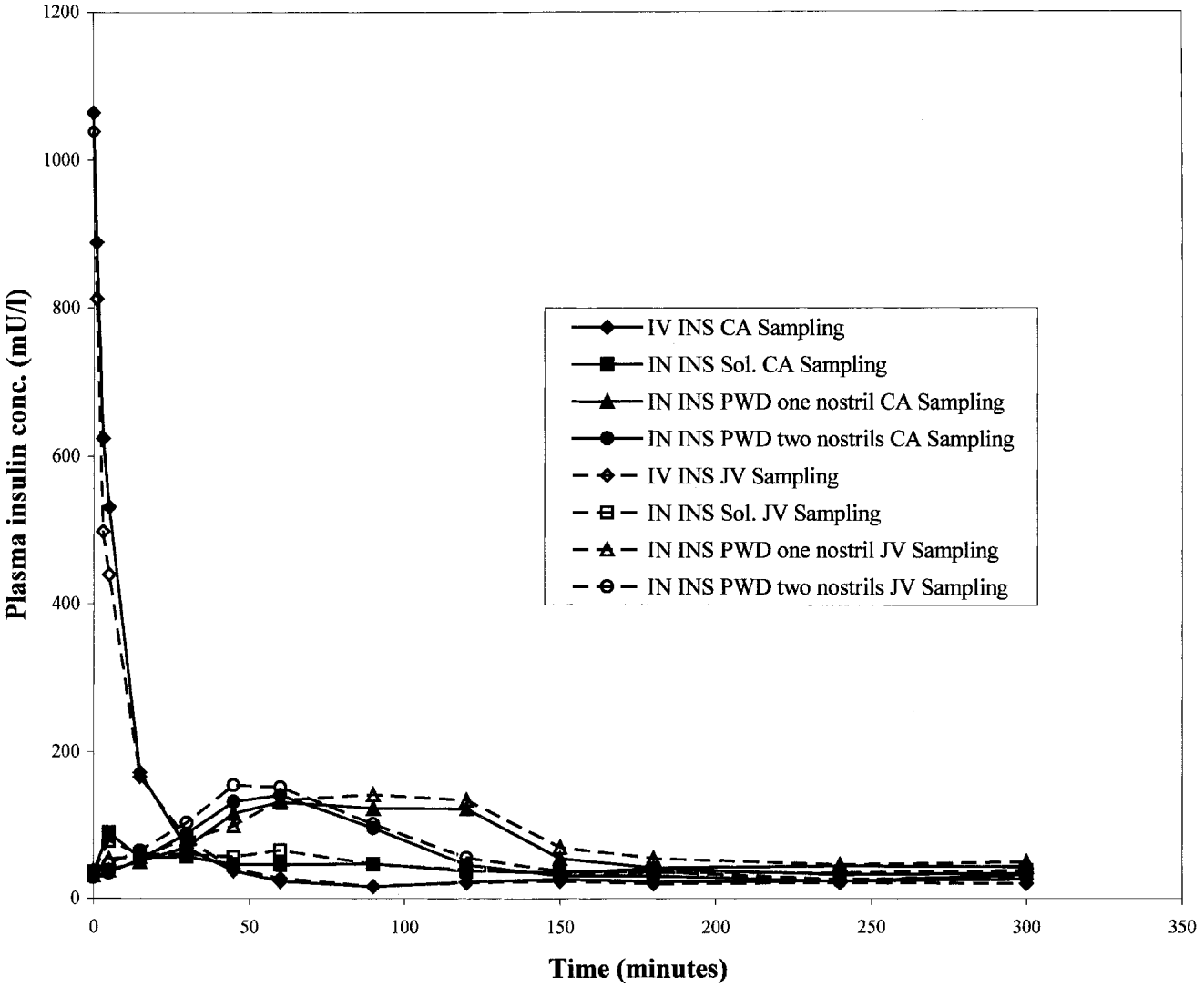

Fig. 2. Plasma insulin–time profiles after administration of insulin formulations by the intravenous and nasal routes to sheep and sampling of blood from the jugular vein and the carotid artery (n = 3).

Table IV. Pharmacokinetic Parameters (±SD) for Insulin Administered by Intravenous Injection and by Nasal Administration to Sheep (n = 3)

Formulation	T_{max} (min)	C_{max} (mU/l)	$C_{max(extrap)}$ (mU/l)	AUC (mU/l·min)	F_{ab} (%)
IV ins CA SAM	1 (0)^{NT}	888.8 (89.6)	1063.9 (159.4)	15961 (3912)	100
IV ins JV SAM	1 (0)^{NT}	812.8 (87.3)	1038.4 (119.6)	15140 (3257)	100
IN ins SOL CA SAM	37 (46)	97.5 (65.3)	NA	12648 (3117)	4 (1)
IN ins SOL JV SAM	42 (32)	94.7 (40.9)	NA	13323 (2364)	4 (1)
IN ins PWD CA SAM (one nostril)	65 (23)	149.9 (57.3)	NA	21979 (7850)	7 (3)
IN ins PWD JV SAM (one nostril)	110 (62)	174.9 (54.0)	NA	23352 (7476)	8 (3)
IN ins PWD CA SAM (two nostrils)	55 (9)	142.0 (82.6)	NA	16673 (5372)	5 (2)
IN ins PWD JV SAM (two nostrils)	50 (9)	157.5 (78.4)	NA	18434 (5647)	6 (2)

Paired *t*-test.
NA, not applicable.
NT, not tested.
For all other pairings, no significant differences indicated ($p > 0.05$).
IV ins CA SAM: intravenous insulin, carotid artery sampling.
IV ins JV SAM: intravenous insulin, jugular vein sampling.
IN ins SOL CA SAM: intranasal insulin solution, carotid artery sampling.
IN ins SOL JV SAM: intranasal insulin solution, jugular vein sampling.
IN ins PWD CA SAM (one nostril): intranasal insulin powder, carotid artery sampling.
IN ins PWD JV SAM (one nostril): intranasal insulin powder, jugular sampling.
IN ins PWD CA SAM (two nostrils): intranasal insulin powder, carotid artery sampling.
IN ins PWD JV SAM (two nostrils): intranasal insulin powder, jugular vein sampling.

listed in Table V. Following intravenous dosing, the plasma concentration–time profiles were comparable for the two sampling sites (Fig. 3). This is also supported by the pharmacokinetic (PK) and statistical findings (Table V); there were no significant differences ($p > 0.05$) between values of C_{max}, $C_{max(extrap)}$, and AUC calculated from the jugular vein and carotid artery data. In contrast, following nasal administration of the morphine–chitosan powder formulation, the plasma drug concentrations, when blood was collected from the jugular vein, were substantially higher than those after sampling from the carotid artery (Fig. 3). There were also statistically significant findings; values of C_{max} and AUC were signifi-

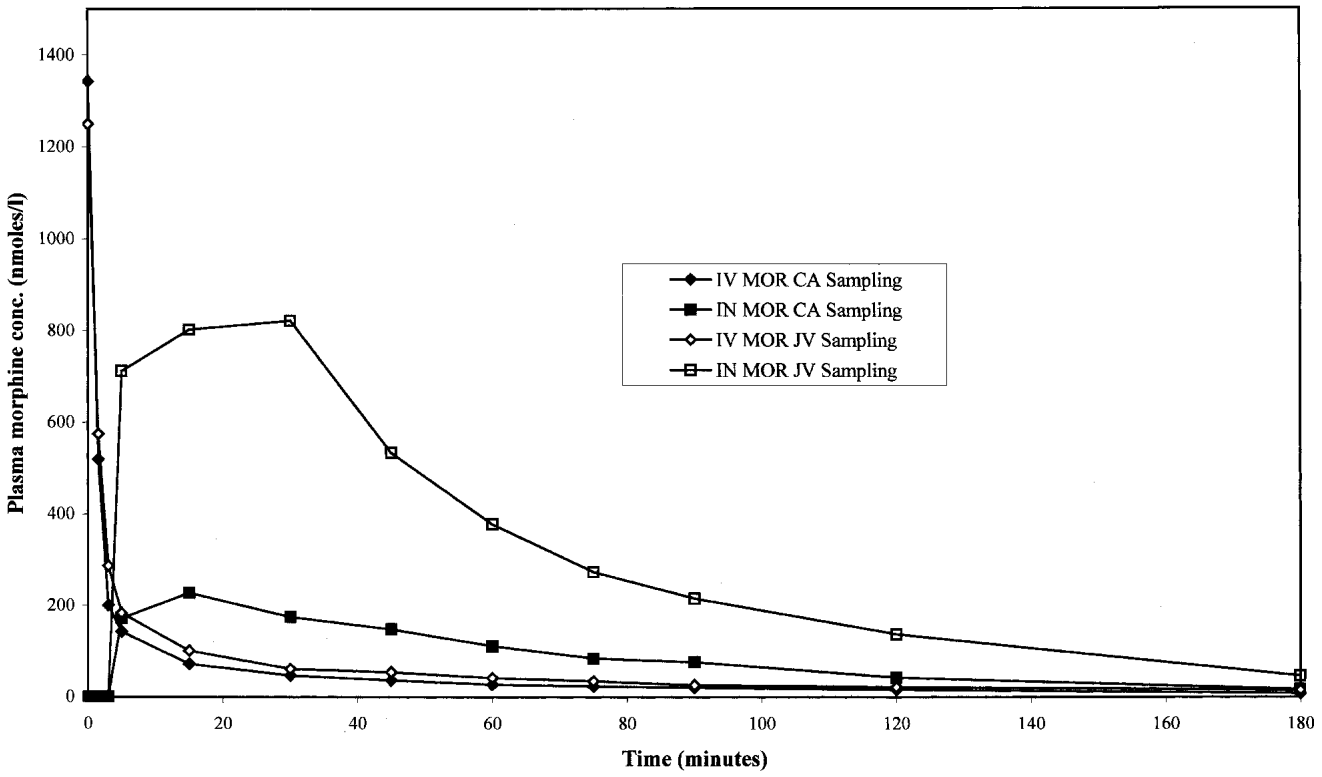

Fig. 3. Plasma morphine–time profiles after administration of morphine formulations by the intravenous and nasal routes to sheep and sampling of blood from the jugular vein and the carotid artery (n = 3).

Table V. Pharmacokinetic Parameters (±SD) for Morphine Administered by Intravenous Injection and by Nasal Administration to Sheep (n = 3)

Formulation	T_{max} (min)	C_{max} (nmole/L)	$C_{max(extrap)}$ (nmole/L)	AUC (nmole · min/L)	F_{ab} (%)
IV MOR CA SAM	1.5 (0)^{NT}	519.6 (111.8)	1,342.9 (314.2)	7,322 (949)	100
IV MOR JV SAM	1.5 (0)^{NT}	575.0 (303.2)	1,249.3 (1097.9)	9,547 (926)	100
IN MOR CA SAM	15 (0)	228.0 (38.3)^{a}	NA	16,036 (1,694)^{b}	73 (8)
IN MOR JV SAM	20 (9)	1,060.4 (559.1)^{a}	NA	57,819 (26,926)^{b}	202 (94)

Paired *t*-test.
NA, not applicable.
NT, not tested.
Significant differences indicated as follows: $^{a,b}p < 0.05$; for all other pairings, no significant differences indicated ($p > 0.05$).
IV MOR CA SAM: intravenous morphine, carotid artery sampling.
IV MOR JV SAM: intravenous morphine, jugular vein sampling.
IN MOR CA SAM: intranasal morphine, carotid artery sampling.
IN MOR JV SAM: intranasal morphine, jugular vein sampling.

cantly higher ($p < 0.05$) when blood was sampled from the jugular vein (1060 nmole/L and 57,819 nmole.min/L, respectively) compared to the carotid artery (228 nmole/L and 16,036 nmole.min/L, respectively) (Table V). The resultant apparent bioavailabilities after carotid artery and jugular vein blood sampling were 73 ± 8% and 202 ± 94%, respectively.

Nicotine

The plasma nicotine data obtained following intravenous and intranasal dosing and blood sampling from the jugular and cephalic veins are represented in Table VI and Fig. 4. Following intravenous dosing, the differences (in C_{max}) observed between the jugular vein and cephalic vein plasma concentration–time profiles were greater than had been observed for morphine and insulin (Fig. 4). There were also significant differences ($p < 0.01$) in terms of values of AUC when samples were obtained from the two different sampling sites (Table VI); values of AUC calculated from cephalic vein and jugular vein data were 673 ng·min/ml and 932 ng·min/ml, respectively. However, differences in plasma concentration and PK data for the two different sampling sites were much greater following nasal dosing (Table VI, Fig. 4). After dosing of the nasal nicotine solution, a substantial elevation in plasma drug concentrations for blood collected from the jugular vein was found. Values of C_{max}, AUC, and F_{ab} were significantly greater ($p < 0.001$–0.01) when blood was sampled from the jugular vein as compared to the cephalic vein. Furthermore, the time to reach peak levels of drug (T_{max}) was significantly shorter ($p < 0.01$) following jugular vein sampling.

For the nasal nicotine–Amberlite powder formulation, plasma drug concentrations in the jugular vein samples were again much higher compared to the concentrations after cephalic vein sampling. Furthermore, differences between values of C_{max}, AUC, and F_{ab} were significant ($p < 0.01$–0.05). The resultant apparent bioavailabilities after cephalic vein and jugular vein blood sampling were 41 ± 17% and 105 ± 37% for the nasal nicotine solution and 121 ± 25% and 244 ± 63% for the nasal nicotine–Amberlite powder, respectively. The bioavailabilities for the two nasal nicotine formulations were found to be close to twofold higher after sampling from the jugular vein as compared to sampling from the

Table VI. Pharmacokinetic Parameters (±SD) for Nicotine Administered by Intravenous Injection and by Nasal Administration to Sheep (n = 5)

Formulation	T_{max} (min)	C_{max} (ng/ml)	$C_{max(extrap)}$ (ng/ml)	AUC (ng · min/ml)	F_{ab} (%)
IV NIC CV SAM	3 (2)^{NT}	22.9 (8.9)^{NT}	57.3 (43.4)	673 (152)^{d}	100^{NT}
IV NIC JV SAM	1 (0)^{NT}	43.5 (5.4)^{NT}	53.9 (8.6)	932 (172)^{d}	100^{NT}
IN NIC SOL CV SAM	16 (9)^{a}	5.0 (2.0)^{b}	NA	306 (123)^{e}	41 (17)^{g}
IN NIC SOL JV SAM	3 (1)^{a}	61.5 (16.3)^{b}	NA	975 (343)^{e}	105 (37)^{g}
IN NIC PWD CV SAM	84 (25)	19.5 (5.2)^{c}	NA	2518 (517)^{f}	121 (25)^{h}
IN NIC PWD JV SAM	63 (40)	76.2 (18.8)^{c}	NA	7028 (1799)^{f}	244 (63)^{h}

Paired *t*-test.
NA, not applicable.
NT, not tested.
Significant differences indicated as follows: $^{h}p < 0.05$; $^{a,c,d,e,f,g}p < 0.01$; $^{b}p < 0.001$. For all other pairings, no significant differences indicated ($p > 0.05$).
IV NIC CV SAM: intravenous nicotine, cephalic vein sampling.
IV NIC JV SAM: intravenous nicotine, jugular vein sampling.
IN NIC SOL CV SAM: intranasal nicotine solution, cephalic vein sampling.
IN NIC SOL JV SAM: intranasal nicotine solution, jugular vein sampling.
IN NIC PWD CV SAM: intranasal nicotine powder, cephalic vein sampling.
IN NIC PWD JV SAM: intranasal nicotine powder, jugular vein sampling.

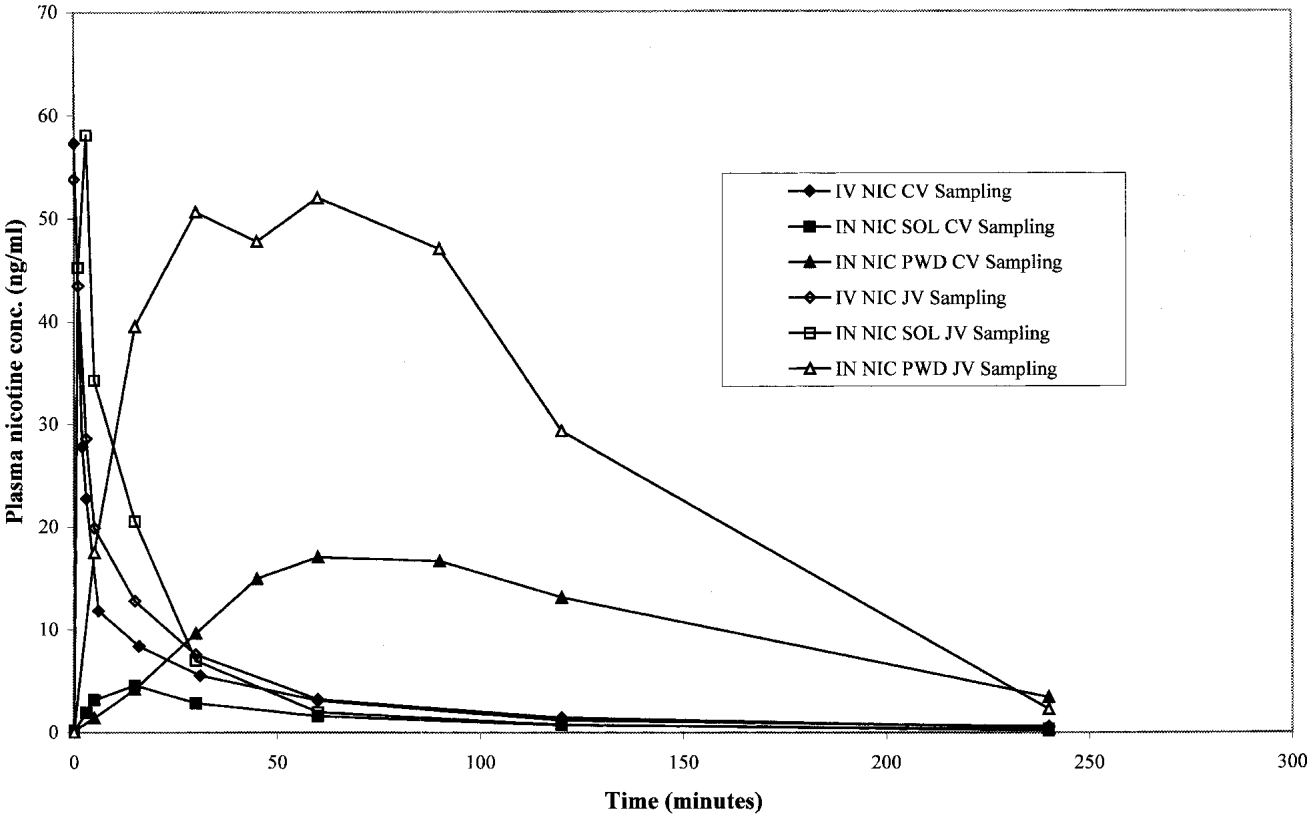

Fig. 4. Plasma nicotine–time profiles after administration of nicotine formulations by the intravenous and nasal routes to sheep and sampling of blood from the jugular and the cephalic veins (n = 3).

cephalic vein. It is also evident that the shape of the plasma concentration vs. time profiles were very different for the powder formulation (sustained increase in plasma levels) from those for the solution formulation. The plasma profiles obtained after jugular vein and cephalic vein sampling following nasal nicotine solution administration show a pulsed delivery (i.e., rapid absorption and rapid decline in plasma nicotine). It should also be noted that the difference in C_{max} and AUC values for the two sampling sites were less for the powder formulation (ratio C_{max}, 3.9; AUC, 2.8) compared to the solution formulation (ratio C_{max}, 12.3; AUC, 3.2).

Computer Simulations

A result obtained from the computer simulations is shown in Fig. 5. The generated profiles for a model drug [volume of distribution (V_d) central, 4 L; V_d deep, 10 L] fit well with the experimental profiles. It can be seen that jugular vein sampling results in a much higher C_{max} (~1.6 μg/ml) than for the carotid artery sampling (~0.8 μg/ml) and a similarly higher AUC (8.2 μg/ml·h) as compared to 6.2 μg/ml·h, respectively.

DISCUSSION

The sheep has long been recognized as a very suitable animal model for the evaluation of pharmacokinetic and pharmocodynamic parameters of a variety of drugs, especially after parenteral administration. In the last 15 years it has been shown that the sheep is also a well suited, relatively inexpensive, and reliable model for nasal absorption studies as evidenced in a number of published papers (6,7,9,14–19). Furthermore, it has been shown for insulin and calcitonin and other selected peptides and small polar drugs that after nasal administration, the results obtained in the sheep model can safely and reliably be extrapolated to man (20).

The morphologic structures in the sheep nasal cavity differ from those of man. The nasal cavity is longer (18 cm), the average surface area is 327 cm^2, and the average volume is 114 cm^3, as compared to 8 cm, 181 cm^2, and 19 cm^3, respectively, in man. However, on a basis of surface area per kilogram body weight the ratio of 8.2 in sheep is similar to that in

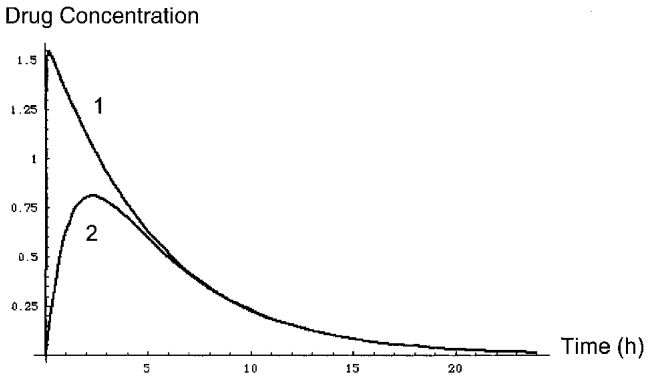

Fig. 5. Computer-simulated plasma–time profiles after simulated nasal administration of a model drug and sampling of blood from the jugular vein and the carotid artery. 1, Jugular vein sampling; 2, Carotid artery sampling.

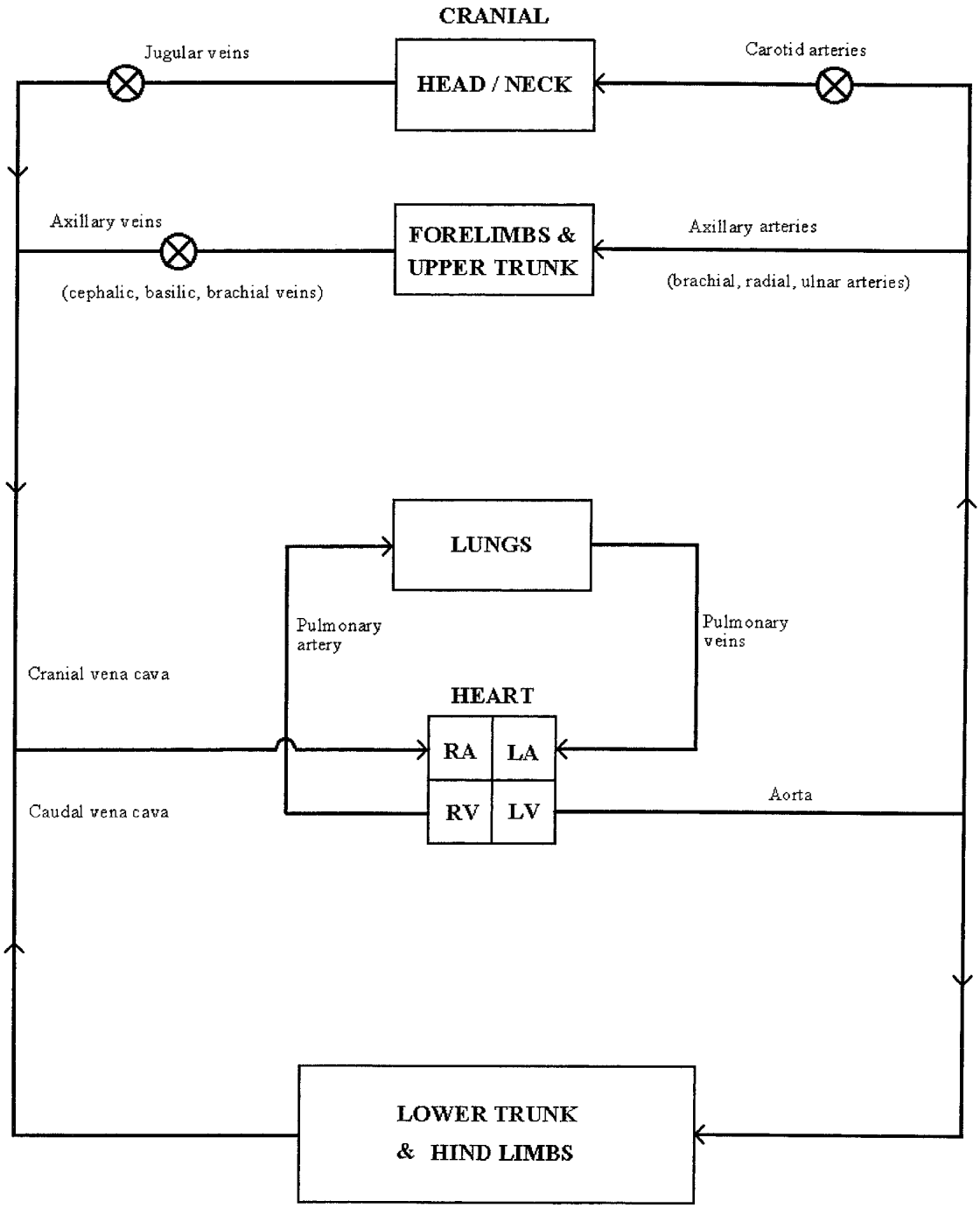

Fig. 6. Diagram of the circulatory system in the sheep showing the relative positions (not to scale) of the jugular vein and carotid artery blood sampling sites.

monkey 7.7 cm^2/kg) and is more similar to that in man (2.5 cm^2/kg) than for most other laboratory animal models available (17). Furthermore Soane *et al.* (21,22) have shown that the nasal mucociliary clearance characteristics of simple and bioadhesive solutions as well as powders are comparable in sheep and in man, thus highlighting physiologic similarities.

Conventionally, in all studies so far reported on the nasal delivery of drugs using the sheep model, the sampling of blood (for the determination of pharmacokinetic or pharmacodynamic parameters) has been performed by sampling blood from the jugular veins either by direct venipuncture or through a sited cannula. Generally, in order to prevent cross-contamination cannulation or venipuncture has been done in the contralateral jugular vein to that used for any form of intravenous injection.

Following nasal administration, a drug will be absorbed into the blood contained in the dense network of capillaries situated in the lamina propria of the nasal mucosa, which is drained via the sphenopalatine foramen into the pterygoid plexus or via the superior ophthalmic vein into the jugular vein. From here the drug will pass to the systemic circulation and into the main body, reaching the liver and the pulmonary

circulations and eventually be carried back toward the upper part of the body, reaching the head via the carotid arteries as depicted in Fig. 6.

Circulatory mixing of the drug is usually assumed to be instantaneous because of the rapid convectional transport by blood flow. Thus, in terms of traditional pharmacokinetics, the blood is thought of as a homogeneous compartment from which the drug may distribute into additional compartments that are ascribed to the various body tissues and fluids (and also to binding to plasma proteins), according to its "volume of distribution" (V_d) (Fig. 1). V_d describes the relationship between the amount of drug in the body and its plasma concentrations and may be defined as a proportionality factor that, when multiplied by the concentration of drug in the blood, yields the total amount of drug present in the body, i.e.,

$$D_t = V_d \times P_t$$

where D_t and P_t represent the total amount of drug in the body and the concentration of drug in the blood at some time, t, respectively (23). The V_d will be dependent on the polarity and molecular size of the drug, with small lipophilic compounds generally showing higher values than more polar drugs, the body weight, which will influence the "compartment" size, and the affinity for protein binding.

Because of the anatomic configuration (Fig. 6) during the phase in which the drug is actively being absorbed across the nasal mucosa (does not apply when only elimination is occurring), there is less likelihood that intravascular mixing and distribution (especially deep compartmental distribution) are complete after jugular vein sampling compared to carotid artery or cephalic vein sampling. Thus, for drugs that tend to be rapidly and extensively absorbed via the nasal route, and for drugs that have a high V_d, this could predictably lead to the measurement of artifactually high plasma concentrations in jugular vein blood samples and hence to an overestimation of bioavailabilities. This is generally the case for drugs with a high octanol/water partition coefficient (at physiologic pH) as opposed to drugs with a low partition coefficient, although researchers have reported that the correlation between adipose storage index and log P can be poor (24). Others have found that it was possible to predict the resultant blood level concentrations of a drug on the basis of knowing the protein binding and the apparent partition coefficient (25).

Apparent blood, plasma, or serum levels of drug may also be influenced by a number of other factors such as whether the plasma assay can determine total drug in the plasma or whether it discriminates between bound or free drug, in which case the extent of plasma protein binding can also affect measured drug levels.

Blood sampling site had no significant effect on the pharmacokinetics of insulin after intravenous or intranasal administration in the sheep. In contrast, there were significant sampling site differences following intranasal but not intravenous dosing of morphine and following both intravenous and intranasal dosing of nicotine to sheep. Where differences were observed, plasma drug concentrations and resulting values of C_{max}, AUC, and bioavailability were much higher in jugular vein samples than following carotid artery (morphine) or cephalic vein (nicotine) sampling. The sampling site had a major impact on the apparent bioavailabilities of morphine and nicotine after nasal dosing. For intranasal morphine, values calculated from the jugular vein data were almost threefold higher than those estimated from the carotid artery data. For intranasal nicotine liquid and powder, there was approximately a twofold difference between values of bioavailability that were estimated from jugular and cephalic vein data. It is interesting to note that the effect of the sampling site diminishes when a nasal nicotine powder formulation is administered as compared to a nasal nicotine solution formulation. This can be explained as an effect of the slower transport of the drug across the nasal mucosa and hence a lower initial drug concentration at the jugular vein sampling site when a controlled-release bioadhesive formulation is used, as evidenced also in the plasma profiles.

Differences in measured drug levels in samples collected from the carotid artery/cephalic vein and jugular vein following intranasal dosing are primarily attributed to relatively incomplete circulatory mixing at the latter site (at least at the earlier sampling times) and thus to an incomplete distribution of the drug throughout the blood together with an incomplete distribution of the drug from the central (blood) compartment to the deep compartments. Thus, it is expected that the effects of noninstantaneous circulatory mixing will become more pronounced as the rate and extent of absorption are increased and that the effect of the noncomplete distribution to the deep compartments will be more pronounced with an increased apparent V_d. The above sampling site differences would also be expected following intravenous administration if blood was taken before sufficient time for the drug to distribute fully throughout blood had elapsed. Thus, conventionally blood samples do not tend to be taken any earlier than about 1 min after completion of the intravenous "bolus" dosing.

The intranasal absorption of insulin via the nasal route is slow and relatively poor. Furthermore, the V_d of insulin is low (in the range of 0.07–0.14 $L \cdot kg^{-1}$) compared to nicotine and morphine (2.6 $L \cdot kg^{-1}$ and 2.6–4.0 $L \cdot kg^{-1}$, respectively), and consequently, there will be less deep compartmental distribution of the insulin from the central (blood) compartment. Thus, the effects of noninstantaneous mixing in the jugular compartment are not evident. With morphine and nicotine, which are absorbed more rapidly and to a greater extent than insulin, and which are distributed more extensively into deeper compartments, sampling site differences are more pronounced following intranasal dosing. For nicotine, of less significance were sampling site differences following intravenous dosing. These differences were really apparent only in samples collected over the first 15 min, which may be a result of regional differences in distribution, although they could in part be related to experimental artifact because blood samples were not taken simultaneously as in the insulin/morphine studies as a result of technical constraints when sampling by venipuncture.

Results demonstrating the significant effect of a blood sampling site on the resultant pharmacokinetic values in a large animal model reported here have not, as far as we are aware, been published before. The results from the computer simulation confirmed the premise that the blood sampling site can affect the resultant plasma concentration–time profile and pharmacokinetic values after nasal administration of drugs in the sheep model. The greater the value of V_d the greater will the effect be on the pharmacokinetic results (all other parameters being constant). Such computer simulations

can be a useful tool when preclinical nasal delivery studies are planned in the sheep model.

It should be emphasized that the effect of blood sampling site on the resultant pharmacokinetic parameters will likewise be applicable to other animal models such as monkey and the dog, especially after nasal administration of drug. Therefore, before any attempt to perform such studies, the characteristics of the drug to be delivered should be carefully considered, and the appropriate blood sampling site selected. Similar effects could be expected from buccal administration and sampling from the jugular vein.

Despite limitations in evaluating drugs that are rapidly and extensively absorbed and that have high V_d, jugular vein sampling in the sheep has proven reliable for ranking the overall performance of nasal dosage forms. Based on previous investigations (data not included here), following administration of a simple intranasal solution of nicotine to sheep with blood sampling via the jugular veins, a linear relationship was demonstrated between nicotine dose and the pharmacokinetic parameters C_{max} and AUC.

CONCLUSIONS

It is evident from the present work that the selection of blood sampling site in an animal model can affect considerably the resultant plasma concentration–time profile and the pharmacokinetic results obtained after nasal delivery of drugs. Three different sampling sites were investigated, namely, the jugular vein that carries blood directly from the head (nasal cavity) to the right side of the heart, the carotid artery that carries blood from the heart to the head region, and the cephalic vein that carries blood from the front extremities to the heart (via the axillary veins and the external jugular veins). Sampling from the jugular vein resulted in an overestimation of plasma drug concentrations and thus bioavailabilities for morphine and nicotine (both are rapidly absorbed and have high V_d values) because the drug would not have been fully distributed through the blood nor to the deep compartments before sampling. When the blood is sampled from the carotid artery or the cephalic vein, respectively, for the two drugs, the plasma levels were lower and most likely closer to the true values. In comparison, for a drug such as insulin, which is poorly/slowly absorbed across the nasal mucosa and that has a very low V_d no sampling site differences (jugular vein vs. carotid artery) were evident, indicating the importance of drug characteristics such as lipophilicity and V_d. Hence, before attempting to perform nasal studies in the sheep model, or indeed in for example the dog or the monkey, the properties of the drug and the drug sampling site should be carefully considered.

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