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Research Paper

Characterization of the Physiological Spaces and Distribution of Tolbutamide in the Perfused Rat Pancreas

Kent John Fanning[1] and Michael S. Roberts[1,2]

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Purpose. To set up and validate a viable perfused rat pancreas model suitable for pharmacokinetic studies.
Materials and methods. We setup and conducted multiple indicator dilution studies in the single pass perfused rat pancreas. The distribution of the reference markers [^{99m}Tc]-red blood cells (RBC), [^{14}C]-sucrose, and [^{3}H]-water, and tolbutamide were analysed using both non-parametric and parametric methods.
Results. The perfusion preparation was observed to be viable by oxygen consumption, outflow perfusate pH, lactate release and insulin release in response to glucose. Parametric analysis of the outflow profiles suggested that the transport of water and tolbutamide from the vascular space was permeability limited. Parametric and nonparametric estimates of V_d for RBC and sucrose were similar and were 0.14±0.01, 0.15 ± 0.005 and 0.35 ± 0.01 ml/g. The parametric estimate for water, 1.04 ± 0.05 ml/g was greater than the nonparametric estimate, 0.89 ± 0.02 ml/g. The multiple indicator dilution method V_d of tolbutamide of 0.75 ± 0.08 ml/g was similar to the reported value of 0.73 ± 0.04 ml/g estimated by tissue partitioning studies.
Conclusions. A viable single pass pancreas perfusion model was established and applied to define distribution spaces of reference markers and the distribution kinetics of tolbutamide.

KEY WORDS: distribution kinetics; pancreas perfusion; pharmacokinetics; physiological spaces; tolbutamide.

INTRODUCTION

Pathologies of the pancreas include diabetes mellitus, pancreatitis and pancreatic cancer. The treatment of non-insulin dependent diabetes mellitus involves compounds which can bind to and/or enter endocrine tissue, whereas drug therapy in pancreatitis and pancreatic cancer require compounds that penetrate into the large mass of exocrine tissue.

The pancreas is therefore an organ in which the distribution of drugs is of critical clinical significance.

However, little is known about drug distribution kinetics in the pancreas. Most studies have examined antibiotic distribution in patients suffering pancreatitis (for review see (1)), with the limited work undertaken on the physiological pharmacokinetics in this organ being limited to *in vivo* partitioning studies (2–4) and the transport of amino acids (5–12). First-pass multiple indicator dilution perfusion models have been utilised to examine the efflux kinetics of numerous solutes in other organs including the liver (13), heart (14), lung (14), kidney (14), hindlimb (15) and head (16). However, to our knowledge, no published studies have yet examined the disposition kinetics of drugs in the perfused pancreas using a multiple indicator dilution technique.

In this work, we describe the setting up and validation of a perfused pancreas model. We then apply the model to examine distribution kinetics of reference substances which define the vascular, extravascular, extracellular and cellular spaces. Finally, we characterise the physiological pharmacokinetics of tolbutamide, a model drug for treating non-insulin dependent diabetes mellitus.

MATERIALS AND METHODS

Preparation of Animals

Male Wistar rats weighing 190–270 g were fed a commercial diet and water *ad libitum*. All procedures involving the animals were carried out with adherence to the University of Queensland Animal Care Committee guidelines (AEC#: MED/249/05/UQ). Following fasting for 16–20 h, animals were anaesthetized by interperitoneal injection of 80 mg/kg ketamine (Parnell Laboratories, Sydney, Australia) and 10 mg/kg xylazine (Bayer, Sydney, Australia).

[1] Department of Medicine, Princess Alexandra Hospital, University of Queensland, Woolloongabba, Queensland 4102, Australia.
[2] To whom correspondence should be addressed. (e-mail: mroberts@soms.uq.edu.au)

ABBREVIATIONS: BSA, bovine serum albumin; MOPS, 3-[*N*-morpholino]propane-sulphonic acid; RBC, red blood cells.

Surgical Procedure

The pancreas and upper duodenum were perfused via the celiac and superior mesenteric trunks of the aorta using a surgical procedure modified from a previously described approach (17). In brief, following laparotomy, the pancreas was separated from surrounding organs and connective tissue by cutting with scissors and blunt dissection with saline-moistened cotton wool applicators. All branches of the celiac and superior mesenteric trunks were tied off except for those travelling directly to the pancreas and upper duodenum. In the method published by Malaisse *et al.* (1990), the upper duodenum was tied off from the pancreas. The duodenum was not separated from the pancreas in the present study, due to the process leading to a long surgical time, handling of the pancreas and potential impairment of viability. The duodenum was cannulated to allow for collection of duodenal fluid during the perfusion. Heparin (David Bull Laboratories, Mulgrave, Australia) was administered prior to cannulation of the aorta. The portal vein was cannulated and the animal killed by thoracotomy.

Perfusion

Once the arterial cannula was positioned in the aorta, perfusion of the preparation began. The perfusion medium was a 3-[*N*-Morpholino]propane-sulphonic acid (MOPS)-buffered Ringer's solution containing (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 (0.9). The medium was supplemented with 4% dextran (60,000–90,000 MW, Sigma-Aldrich, St Louis, USA) and 0.5% bovine serum albumin (BSA) (Thermo, New Zealand). In order to increase the oxygen carrying capacity of the perfusate, 20% (volume/volume) prewashed canine RBC were added in certain perfusions. The perfusion apparatus consisted of a perfusate reservoir, peristaltic pump (Masterflex L/S standard drive system, Cole-Parmer, IL), artificial lung (silastic tubing in an oxygen rich humidifier), bubble trap, water manometer and water-jacket. The perfusate was pumped through silicone tubing (Masterflex 96400-14, Cole-Parmer, IL). Heating of the reservoir and the presence of the water-jacket, which enclosed the tubing adjacent to the inflow cannula, maintained the temperature of the inflowing perfusate at 37°C. The pump was adjusted to achieve a flow rate of ~2 ml/min from the outflow cannula. Each perfusion included an equilibration phase that lasted 15 min, in which stable and suitable flow and pressure were established.

Multiple Indicator Dilution Studies

The three reference markers used were, [^{99m}Tc]-RBC (800 mBq/ml, the ^{99m}Tc was obtained from the Nuclear Medicine Department, Princess Alexandra Hospital, Brisbane, Australia, and labelling of RBC was undertaken using an UltraTag RBC Kit, Mallinkrodt Medical, USA), [^{14}C]-sucrose (25 μCi/ml, Perkin Elmer, >97% purity) and [^{3}H]-water (50 μCi/ml, Perkin Elmer). Following the equilibration phase, reference markers were co-administered with a Hamilton syringe in a single 20 μl bolus injection, via an injection port attached to the inflow cannula. A programmed fraction collector, specially constructed using a stepper motor and driver (to allow for sampling up to 0.2 s), was started simultaneously with the injection. Thirty five fractions were collected over a 7 min period. Fractions were normally collected at the following times, background, 3.6, 4.8, 6, 7.2, 8.4, 9.6, 10.8, 12, 13.2, 14.4, 16.8, 19.2, 21.6, 24, 26.4, 30, 33.6, 37.2, 40.8, 48, 55.2, 62.4, 69.6, 76.8, 91.2, 105.6, 120, 148.8, 177.6, 206.4, 242.4, 278.4, 314.4, 350.4, 386.4 s. In the perfusions using RBC-free medium, [^{99m}Tc]-RBC was not included in the bolus.

Similar experiments were undertaken with a 20 μl bolus injection containing [^{3}H]-water and [^{14}C]-sucrose, followed by a 20 μl bolus injection of tolbutamide (2 mg/ml, Sigma-Aldrich, St. Louis, USA) 10 min later.

A study was also conducted to determine the transit time density function of the catheters, in which a 20 μl bolus injection containing [^{14}C]-sucrose was injected with the catheters joined together, without the preparation present.

Pancreas Preparation Physiological and Biochemical Viability

The viability of the pancreas perfusion was assessed over time by measuring a number of parameters. Flow rate was measured by weighing the amount of fluid leaving the outflow cannula over a 30 s period. The flow rate coming into the organ preparation via the inflow catheter was also measured at the end of the experiment, following removal from the aorta. The percent leakage was calculated by using the formula, (1 - outflow flow rate/inflow flow rate)×100. The perfusion pressure was read from the height of the fluid in the manometric-tubing. An ABL520 blood gas analyser (Radiometer, Copenhagen) was used to measure the pH, pO_2 and (in the perfusions using RBC-containing medium) hemoglobin (Hb) saturation in samples from the inflow and outflow cannulas. The following formula was used to determine the oxygen consumption of the preparation.

$$O_2 \text{ consumption} = (pO_{2in} - pO_{2out}) \times S \times Q / \text{preparation weight(g)},$$

where $S = O_2$ solubility factor (1.1699 μmol/l Torr), Q = flow rate (l/min).

In perfusions where RBC were present in the perfusate, the HbO_2 consumption was added to the dissociated oxygen consumption to calculate the total oxygen consumption.

$$HbO_2 \text{ consumption} = ([O_2]\text{in} - [O_2]\text{out}) \times Q \times 0.44623 / \text{preparation weight(g)},$$

where 0.44623 is the conversion factor from ml O_2 in 100 ml to mmol·l^{-1} O_2 (18).

In several perfusions the insulin-releasing ability of the preparation was examined. Outflow samples were collected on ice prior to, and then at, 1 min intervals during infusion of 2.9 g/l glucose or 0.1 g/l tolbutamide. Samples were centrifuged at 3,000 rpm for 10 min and the supernatant was removed and stored at −20°C. In the perfusions where glucose was infused, insulin content was determined using a commercially obtained rat insulin ELISA kit (Linco, Missouri, USA), while for the samples collected during the

tolbutamide infusions, a rat insulin RIA kit (Linco, Missouri, USA) was used. In several perfusions, samples were also measured for lactate using a 747 Autoanalyser (Hitachi, Japan). Following the completion of perfusion, the perfused preparation was removed from the animal and weighed. After drying for 72 h at 50°C, the preparation was again weighed and the dry/wet ratio calculated.

Sample Analysis

The radioactivity of samples collected after injection of [^{99m}Tc]-RBC was recorded immediately using a Cobra 2 gamma counter (Packard, Meriden, USA) and corrected as necessary for radioactive decay over time. Samples were then stored for 3 days, during which the ^{99m}Tc decayed to less than 10^{-4} initial counts. These samples were then counted for ^{3}H and ^{14}C by adding 20 µl of supernatant of each sample to 2 ml scintillation fluid and counting using a Tri-Carb 2700TR scintillation counter (Packard, Meriden, USA). The injection mixture was diluted 1:50 in the perfusion medium, prior to counting by the same procedure as perfusate samples.

The samples collected following bolus injection of tolbutamide were assayed by HPLC using a protein precipitation procedure in which 100 µl of sample was added to 100 µl of acetonitrile and 50 µl of internal standard (chlorpropamide 10 µg/ml in acetonitrile). After vortexing and centrifugation at 10,000 rpm for 5 min, 10 µl of each sample was injected onto a C_{18} column (Waters Symmetry) with a mobile phase of 40% acetonitrile / 60% water (pH=3, using phosphoric acid) at a flow rate of 1 ml/min with detection at 230 nm. The HPLC system consisted of a SCL-10A XL auto injector (Shimadzu), SCL-10A VP system controller (Shimadzu), LC-10AT liquid chromatograph (Shimadzu) and a SPD-10AV UV-VIS detector (Shimadzu). Calibration curves were linear between 0.5 and 100 µg/ml, with r^2 values > 0.999. The within-day coefficient of variation was 0.9–1.1%, the recovery was 103 ± 1%, and the limit of detection was 0.1 µg/ml.

Data Analysis

The raw radioactive counts (CPM—^{99m}Tc) or disintegrations (DPM—^{3}H and ^{14}C) of all samples were subtracted from the counts/disintegrations in the background samples. The resultant data was then analysed using a two phase organ model of the pancreas after correction for catheter effects. RBC were used to characterize the vascular space and large vessel effects, in a similar fashion to that originally described by Chinard *et al.* (19) and later by Goresky *et al.* (20–23). The large vessel volume was calculated by multiplying the lag time for the RBC (corrected by subtraction from the lag time due to the catheter) by the flow rate. The two-phase model used was similar to that previously described for hepatic disposition (24) in that it assumed a heterogeneity in transit times through the vascular space (vascular dispersion) and transport across a permeability barrier between the vascular and tissue spaces. A sum of two inverse Gaussians in the Laplace domain was used as the function to describe both catheter dispersion and organ vascular dispersion, its Laplace function $\hat{f}_i(s)$ being given by

$$\hat{f}_i(s) = p\exp\left\{\frac{1}{CV_1^2} - \left[\frac{MT_1}{CV_{1/2}^2}\left(s + \frac{1}{2MT_1CV_1^2}\right)\right]^{1/2}\right\} + (1-p)\exp\left\{\frac{1}{CV_2^2} - \left[\frac{MT_2}{CV_{2/2}^2}\left(s + \frac{1}{2MT_2CV_2^2}\right)\right]^{1/2}\right\} \quad (1)$$

where MT_1 and MT_2 are the mean transit times for the two distributions and CV_1^2 and CV_2^2 are the corresponding variances and p (empirical parameter, estimated by nonlinear regression) is the relative proportion of the two (25). The catheter effect function $\hat{f}_{cath}(s)$ was estimated by nonlinear regression of the catheter [^{14}C]-sucrose outflow-time profile using a weighting of $1/y^2$. The catheter parameters were then fixed ($p = 0.312$, $MT_{1cath} = 4.35$ s, $MT_{2cath} = 11.11$ s, $CV_{1cath}^2 = 0.04$, $CV_{2cath}^2 = 0.48$) in the estimation of the vascular space function $\hat{f}_B(s)$ by nonlinear regression of a sum of two inverse Gaussian density functions for either the [^{99m}Tc]-RBC or [^{14}C]-sucrose outflow profile $C_B(t)$ after injection of a dose Dose into the perfused organ at a flow rate Q, also with a weighting of $1/y^2$:

$$C_B(t) = \frac{Dose}{Q} L^{-1}\left\{\hat{f}_{cath}(s)\hat{f}_B(s)\right\} \quad (2)$$

The mean transit time for a given marker MMT_j and normalized variance CV_j^2 was then estimated using

$$MMT_j = p_j MT_{1B,j} + (1-p)MT_{2B,j} \quad (3)$$

And

$$CV^2 = \frac{p(CV_1^2+1)MT_1^2 + (1-p)(CV_2^2+1)MT_2^2}{MTT^2} - 1 \quad (4)$$

The distribution of water and tolbutamide were then estimated assuming a permeability barrier model in which the solute distributes between the vascular and interstitial space to the cellular space, using vascular and interstitial spaces defined by [^{14}C]-sucrose:

$$C_s(t) = \frac{Dose}{Q} L^{-1}\left\{\hat{f}_{cath}(s)\hat{f}_B\left[s + k_{in}\left(1 - \frac{k_{in}}{k_{in} + s\frac{V_C}{V_B}}\right)\right]\right\} \quad (5)$$

where $C_s(t)$ is the outflow concentration-time profile for [^{14}C]-sucrose, k_{in} is the permeability rate constant from the vascular and interstitial space to the cellular space and V_C and V_B are the cellular and extracellular [vascular + interstitial] spaces. The permeability rate constant from the tissue to the vascular and interstitial space was estimated by: $k_{out} = k_{in}V_B/V_C$. In these regressions, V_B was defined by the MTT of the vascular reference ($MTT_B = V_B/Q$). For water and tolbutamide the MTT and CV^2 were defined by

$$MTT = \frac{V_B + V_t}{Q} \quad (6)$$

where V_t is the tissue distribution volume, and

$$CV^2 = CV_B^2 + \frac{Q}{CL_{BT}} \frac{2v^2}{(1+v)^2} \quad (7)$$

where CL_{Bt} is the permeation clearance ($= k_{in} \times V_B$), and $v = \frac{V_C}{V_B}$. CL_{Bt} is related to the fraction of solute unbound in the perfusate, f_{up}, and the permeability surface are product (PS) by the equation $CL_{Bt} = f_{up} \times PS$ (25).

When RBC were present in the perfusate the V_d of sucrose, $V_{sucrose}$, was adjusted to account for the fact that it does not distribute into RBC (24) and was calculated as

$$V_{sucrose} = MTT \times Q\,(1 - \text{hematocrit})/\text{weight of preparation.} \quad (8)$$

The vascular, interstitial, intracellular and extravascular spaces were determined from the RBC, sucrose and water volumes as follows; vascular $= V_{RBC}$, extravascular $= V_{water} - V_{RBC}$, extracellular $= V_{sucrose}$, cellular $= V_{water} - V_{sucrose}$. The recoveries ($F$) and MTTs of the solutes were also estimated by statistical moments using the trapezoidal rule and appropriate corrections for the time period after the last sample:

$$F = \frac{Q\int_0^\infty Cdt}{Dose} = \frac{Q.AUC}{Dose} \quad (9)$$

And

$$MTT = \frac{\int_0^\infty tCdt}{\int_0^\infty Cdt} = \frac{AUMC}{AUC} \quad (10)$$

Perfusate, Pancreatic and Duodenal Binding of Tolbutamide

The fraction of tolbutamide that was unbound in the perfusate, f_{up}, and in the tissue, f_{ut}, was determined using ultrafiltration. In both instances, 1 ml of a final incubation solution was loaded into a Centrifree ultracentrifugal device (Amicon, YM-30) and centrifuged at 1,100 × g for 5 min. The collected filtrate and the incubation solution were then extracted as described for the perfusion samples and similarly analysed by HPLC. The fraction unbound was determined as the concentration of tolbutamide in the filtrate divided by the initial concentration in the incubation solution. The incubation solution for the perfusate consisted of a 20 μg/ml solution of tolbutamide prepared in the RBC-free perfusate, vortexed and incubated at 37°C for 30 min.

The tissue incubation solution was prepared from pancreas and duodenum tissue that was collected from rats following perfusion with cold saline via a cannulated aorta. Homogenisation was undertaken by mincing with scissors, addition of 50 mM tris buffer containing 0.25 M sucrose, use of a Polytron homogenizer and a glass homogenizer, which yielded a 0.2 g/ml pancreas and duodenal homogenate. The protein concentration of the homogenates was determined using a commercial BCA Protein Assay Kit (Pierce, Rockford, IL) with spectrophotometric detection at 540 nm (homogenates had a protein concentration of 15 mg/ml). A 25 μg/ml solution of tolbutamide was prepared in both the pancreas and duodenum homogenates and incubated at 37°C for 30 min. The tissue to perfusate partition coefficient, K_p, was estimated as

$$K_p = f_{up}/f_{ut} \quad (11)$$

where f_{up} is the fraction unbound in the perfusate and f_{ut} is the fraction unbound in the perfused tissue. An estimated volume of distribution, V_d, was then calculated assuming:

$$V_d = V_{plasma} + (f_{up}/f_{ut}) \times V_{tissue} \quad (12)$$

where V_{plasma} is the plasma volume ($=V_{sucrose}$), and V_{tissue} is the cellular water volume ($=V_{water} - V_{sucrose}$) (15,26). The value of f_{ut} was estimated using Eq. (12).

The tissue distribution volume of the unbound solute, $V_{t,u}$, was also estimated using the equation,

$$V_{t,u} = \frac{V_t}{f_{ut}}.$$

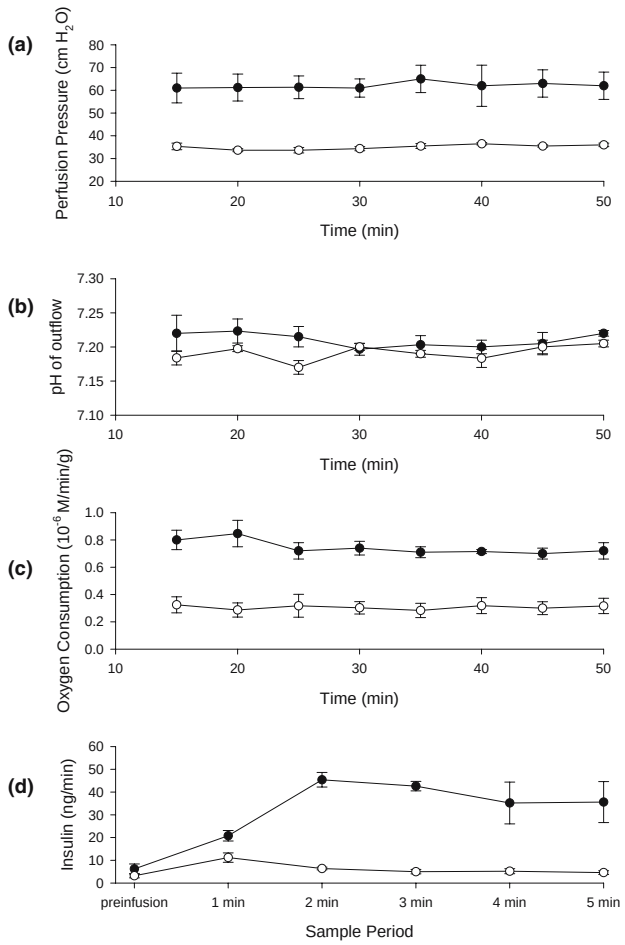

Fig. 1. (a) Perfusion pressure, (b) pH of outflow perfusate and (c) oxygen consumption with time, from perfusions using RBC-free (*empty circle*) and RBC-containing perfusate (*filled circle*). Each data point represents mean ± SEM ($n = 5$). (d) Insulin release by the perfusion preparation during infusion of 2.9 g/l glucose (*filled circle*) and infusion of 0.1 g/l tolbutamide (*empty circle*). Each data point represents mean ± SEM ($n = 3$ for glucose infusion, $n = 4$ for tolbutamide infusion).

Table I. Perfusion Viability Parameters (Mean ± SEM, $n = 5$)

Parameter	RBC-free perfusate	RBC-containing perfusate
Flow (ml/min)	2.0±0.1	2.1±0.04
Pressure (cm H_2O)	35±1	62±6[a]
ΔpH (inflow–outflow)	0.11±0.004	0.08±0.01
Oxygen consumption (μM/min.g)	0.32±0.06	0.77±0.07[b]
Lactate in outflow (mM)	1.13±0.04	n.d.
dry/wet ratio—pancreas	0.27±0.002	0.27±0.003
—whole preparation	0.23±0.004	0.23±0.002

[a] In perfusions where RBC were present in the perfusate, the pressure was significantly higher ($p < 0.03$) than in perfusions where RBC-free perfusate was used.
[b] The oxygen consumption was significantly higher ($p < 0.002$) when RBC-containing perfusate was used.

RESULTS

Perfusion Preparation Viability

Figure 1a–c shows a typical perfusion pressure, pH in the outflow perfusate and oxygen consumption of the preparation *versus* time profile. The various parameters are summarised in Table I. RBC in the perfusate did not affect flow rate but did affect the pressure. The pressure was significantly higher, 62 ± 6 *vs.* 35 ± 1 cm H_2O ($p = 0.026$), in the perfusions that contained RBC in the perfusate. The leakage from preparations in which RBC were or were not in the perfusate was 16 ± 7 and 20 ± 3%, respectively. The presence of RBC in the perfusate also significantly affected the oxygen consumption ($p = 0.002$) of the preparation, with values of 0.32 ± 0.06 and 0.77 ± 0.07 μM/min.g being found for RBC-absent and present in the perfusate.

Insulin was released during infusion of glucose or tolbutamide with a typical profile shown in Fig. 1d. In general, the insulin rate peaked at 2 min after glucose infusion with the overall increase being seven-fold of that of the basal rate. The maximum insulin response to tolbutamide was evident at 1 min following the onset of infusion, having a 3.5-fold higher rate than that observed pre-infusion. Lactate release was consistent over the course of the experiment (data not shown), with levels of 1.13 ± 0.04 mM being evident in the outflow.

The wet weight of the pancreas, following perfusion, was 1.07 ± 0.04 g. The water content of the pancreas and duo-

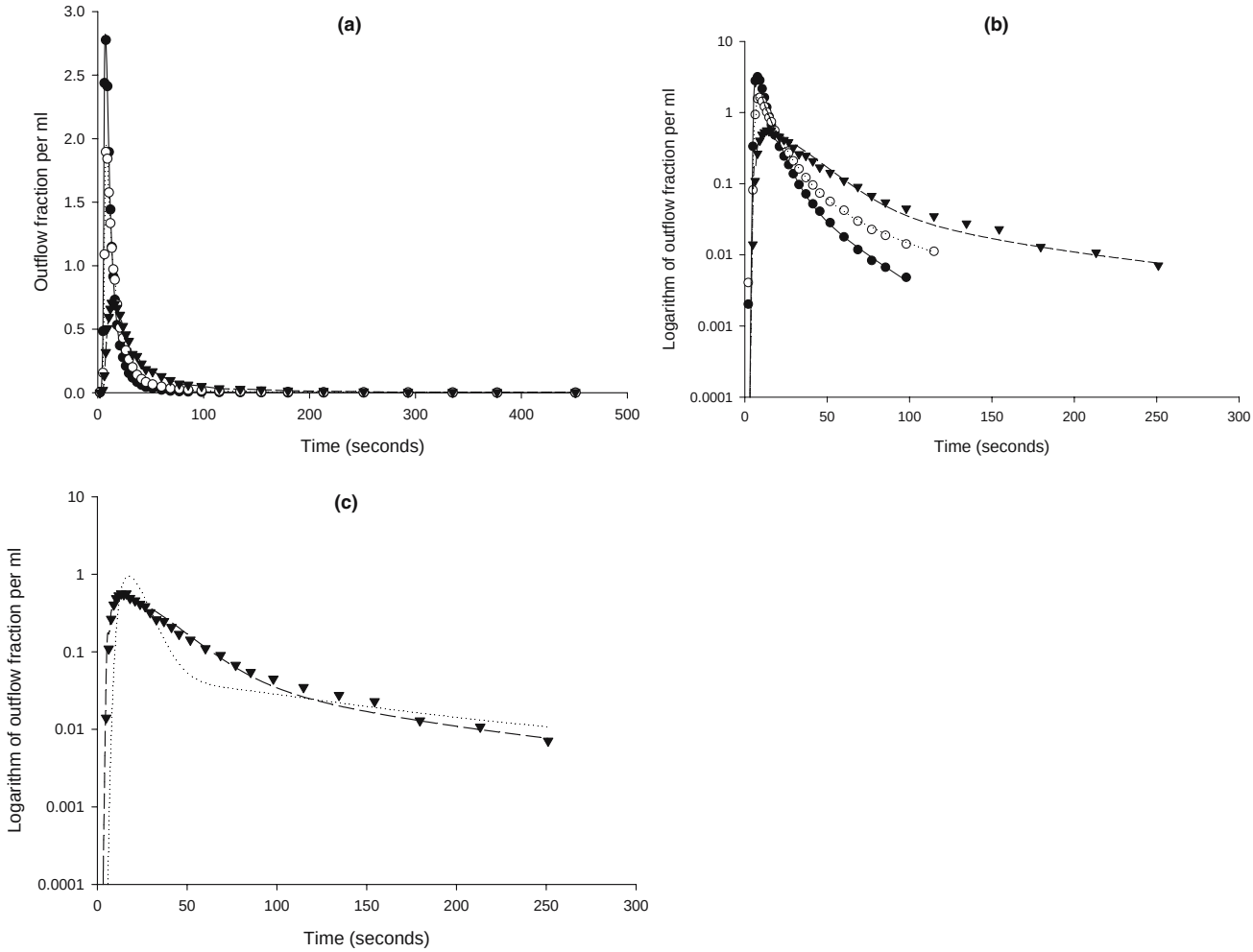

Fig. 2. (a) Typical outflow profile of RBC (*filled circle*), sucrose (*empty circle*) and water (*filled inverted triangle*) shown on a linear scale with data joined point to point. (b) Typical fits, using the 2-phase model, of the same outflow profile data of RBC, sucrose, and water on a log-linear scale. In (b) the *lines* indicate the fitted curves. (c) Typical fit of the outflow profile data of water using the combined space-distributed barrier-limited model (*dashed line*, from (b)) compared with that of the space-distributed model (*dotted line*).

Table II. Non-parametric and Parametric Statistical Moments for the Distribution of Reference Markers and Tolbutamide in the Perfused Rat Pancreas (Mean ± SEM)

	Non-parametric			Parametric	
Tracer	Percent Recovery	MTT (s)	V_d (ml/g)	MTT (s)	V_d (ml/g)
[^{99m}Tc]-RBC (n=5)	84±5	8.1±0.7	0.15±0.005	7.1±0.6	0.14±0.01
[^{14}C]-sucrose (n=11)	81±3	21.2±1.0	0.35±0.01[a]	21.7±1.0	0.35±0.01[a]
[^{3}H]-water (n=11)	79±5	51.2±1.9	0.89±0.02[b]	71.6±4.8	1.13±0.07[b,c]
Tolbutamide (n=4)	74±4	52.5±3.9	0.82±0.12[d]	71.0±2.3	1.04±0.08[d,e]

MTT Mean transit time, V_d volume of distribution.
[a] The V_d of sucrose was significantly larger ($p < 0.00001$) than the V_d of RBC.
[b] The V_d of water was significantly larger ($p < 0.00001$) than the V_d of sucrose and RBC.
[c] The V_d of water obtained by parametric analysis was significantly larger ($p < 0.05$) than the V_d obtained by nonparametric analysis.
[d] The V_d of tolbutamide was significantly larger ($p < 0.002$) than the V_d of sucrose and RBC.
[e] The V_d of tolbutamide obtained by parametric analysis was significantly larger ($p < 0.04$) than the V_d obtained by nonparametric analysis.

denum were 0.79 ± 0.03 and 0.76 ± 0.03 ml, respectively. The dry/wet ratio of the pancreas and the dry/wet ratio of the whole preparations (pancreas and duodenum) were the same regardless of whether RBC were present in the perfusate. The dry/wet ratio of the pancreas and whole preparation were not significantly different from animals that had not been perfused (0.29 ± 0.008 and 0.25 ± 0.009). The hematocrit of RBC-containing perfusate was 0.13 ± 0.005.

Distribution of Reference Markers

Figure 2a shows the linear outflow profile for [^{99m}Tc]-RBC, [^{14}C]-sucrose and [^{3}H]-water. Figure 2b shows the regressions obtained using a space-distributed model only for RBC and sucrose and a combined space-distributed barrier-limited model for water. The latter model was chosen for water as a space-distributed model only poorly described the data shown in Fig. 2c.

The lag time before the appearance of RBC in the perfusate, after bolus injection, occurred at 4.6 ± 0.2 s and the catheter lag time was 2.0 ± 0.1 s. The difference in lag times (4.6–2.0) yielded a large vessel volume of 0.09 ± 0.02 ml. The transit time for RBC (corrected for catheter effects) of 8.1 ± 0.7 s corresponded to a volume of 0.28 ± 0.02 ml so that the capillary volume (based on total RBC volume large vessel volume) was twice as large as the large vessel volume in the perfusion preparation.

Table II contains a summary of the statistical moments and derived volumes from both non-parametric and parametric analysis of the distribution data. As can be seen, the V_d of RBC is significantly less than sucrose ($p < 0.00001$), which is less than that of water ($p < 0.00001$). The parametric and non-parametric results of RBC and sucrose were similar. However the V_d estimated from fitting the water data, 1.13 ± 0.07 ml/g, was significantly larger than that calculated from non-parametric analysis, 0.89 ± 0.02 ml/g ($p < 0.05$). The water outflow profile could not be fitted using RBC as a reference.

The physiological spaces deduced by nonparametric and parametric modelling were respectively, for vascular 0.15 ± 0.005, 0.14±0.01 ml/g; for extravascular 0.75 ± 0.03, 0.89 ± 0.09 ml/g; for extracellular 0.35 ± 0.01, 0.35 ± 0.01; and for cellular 0.56 ± 0.02, 0.81 ± 0.06 ml/g. Significantly larger volumes were estimated by parametric analysis for the extravascular ($p < 0.05$) and cellular space ($p < 0.001$).

The CV[2] of RBC, sucrose and water were similar, being 4.07 ± 0.28, 3.98 ± 0.76 and 4.21 ± 0.75, respectively.

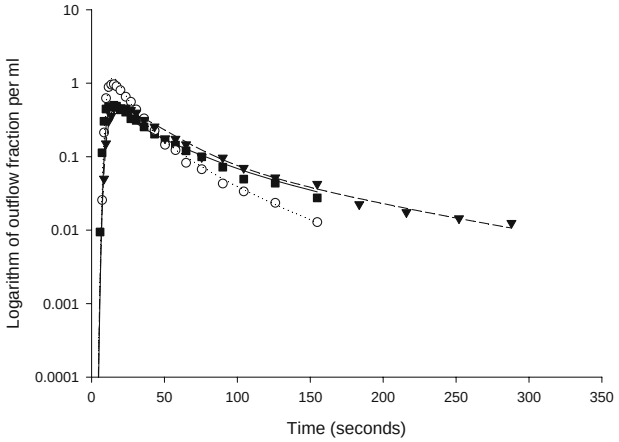

Fig. 3. Typical fit of outflow profile data of tolbutamide (*filled square*) on a log-linear scale, together with sucrose (*empty circle*), used as the reference for the fitting, and water (*filled inverted triangle*), which shows total tissue space. The *lines* indicate the fitted curves.

Distribution of Tolbutamide

Figure 3 shows the experimental outflow data of tolbutamide and the regression obtained using the permeability limited two-phase model together with sucrose, used for the fitting, and water which shows total water space. A space-distributed model for tolbutamide showed a poor fit (data not shown), similar to the plot shown for water (Fig. 2c). The estimated V_d for tolbutamide from both parametric and non-parametric analysis of outflow profiles was significantly larger than sucrose but slightly less than that of water (Table II).

The estimated partition coefficients of the unbound fraction of tolbutamide, for the pancreas, $K_{p,panc}$, and duodenum, $K_{p,duo}$, were 0.50 ± 0.03, and 0.38 ± 0.05, respectively. Using these partition coefficients and the reference marker volumes the estimated V_d was 0.73 ± 0.04 ml/g, similar to 0.82 ± 0.12 ml/g calculated by non-parametric analysis of the outflow profile.

Table III summarizes the uptake kinetics of water and tolbutamide. The k_{in} and k_{out} for water and tolbutamide were 0.27 ± 0.04 and 0.10 ± 0.01 s^{-1}, and 0.11 ± 0.01 and 0.06 ±

Table III. Model Derived Parameter Estimates and Fraction Unbound for Water and Tolbutamide (mean ± SEM, n=11 for water and 4 for tolbutamide)

Parameter or Measured Value	[^{3}H]-Water	Tolbutamide
f_{up}[a]	1.0[g]	0.36±0.01
f_{ut}[a]	1.0[g]	0.80±0.08
k_{in} (s^{-1})[b]	0.27±0.04	0.11±0.01
k_{out} (s^{-1})[b]	0.10±0.01	0.06±0.01
CL_{Bt} (ml/min.g)[c]	5.42±0.66	2.35±0.23
V_t(ml/g)[d]	0.81±0.06	0.67±0.13
$V_{t,u}$[e]	0.81±0.06	0.80±0.08
PS (ml/min.g)[f]	5.42±0.66	6.52±0.63

f_{up} Fraction unbound in perfusate (MOPS buffer, 4% detxran, 0.5% BSA, pH = 7.4), f_{ut} fraction unbound in tissue, k_{in} permeation rate constant, k_{out} efflux rate constant, CL_{Bt} permeation clearance, V_t tissue distribution volume, $V_{t,u}$ tissue distribution volume of the unbound solute.
[a] Measured value.
[b] Estimated parameter by nonlinear regression.
[c] Derived parameter from the equation $CL_{Bt} = k_{in} \times V_B$.
[d] Derived parameter from equation $V_t = V_{sucrose} \times v$.
[e] Derived parameter from equation $V_{t,u} = \frac{V_t}{f_{ut}}$.
[f] Derived parameter from equation $PS = k_{in} \times V_B / f_{up}$.
[g] Water was assumed to have f_{up}, f_{ut} of 1.0.

0.04 s^{-1}, respectively. This yielded CL_{Bt} of 5.42 ± 0.66 and 2.35 ± 0.23 ml/min.g and PS values of 5.42 ± 0.66 and 6.52 ± 0.63 ml/min.g, respectively. The tissue distribution volumes were 0.81 ± 0.06 (water) and 0.67 ± 0.13 ml/g (tolbutamide). The unbound tissue distribution volume,$V_{t,u}$, for tolbutamide was equivalent to water. The estimated fraction unbound in the tissue, obtained from Eq. (12), using the known distribution spaces and f_{up}, was 0.85 ± 0.15. A similar value was found experimentally (0.80 ± 0.08) based on homogenised tissue fraction unbound.

DISCUSSION

Perfusion Preparation Viability

This work established a viable perfused pancreas preparation and used the multiple indicator dilution method to define the distribution kinetics for reference markers and tolbutamide using both non-parametric and parametric methods.

The perfusion preparation consisted of the pancreas and the upper section of duodenum that has continuous vascular connections with the pancreas.

In this work 2 ml/min was found to be the optimal perfusate flow rate. The physiological flow, in rats, has been cited as 0.6 ml/min.g pancreas (27) and 1.3 ml/min.g pancreas (1.6 ml/min.g duodenum) (28). Malaisse *et al.* (1990) has recommended a perfusion rate of 1.6–2 ml/min for rats between 200 and 250 g (17). In studies evaluating the transport of amino acids in the perfused pancreas, flows of approximately 1.8 ml/min have been used (29). In preliminary studies we found that flow rates exceeding 2 ml/min caused oedema (data not shown). Whereas, at flow rates lower than 2 ml/min, insufficient oxygen consumption was observed (data not shown). The perfusion pressures observed during our studies of 32–80 cm H_2O, accompanying flow rates of ~2 ml/min, were similar to values (40–90 cm H_2O) that were thought to achieve maximal vasodilation of the vasculature (30). Elevations in perfusion pressure, to a similar extent to what was observed in the pancreas perfusions, when using RBC-containing perfusate *versus* RBC-free perfusate, have been previously observed in liver perfusions undertaken in our laboratory (31).

The oxygen consumption of the preparation in RBC-free conditions was similar to that observed in perfusions by Lenzen (1979) (0.3 μM/min) (32). When RBC were used to supplement the medium, the oxygen consumption observed was greater than that seen previously at a similar perfusion flow (0.53 ± 0.08 μM/min.g) (8) and equal to that observed at significantly higher flow rates (0.78 μM/min.g) (32), where RBC were not present.

The time course and magnitude of insulin release during glucose infusion was similar to that seen in previous studies (17,33,34). Likewise, the release profiles during tolbutamide infusion replicated past observations in the perfused pancreas (33,35,36). The small drop in pH (~0.1 pH unit) across the preparation, observed in this work, is similar to that previously shown in viable pancreas preparations (8,32).

In this study, a goal was to maintain lactate at ~1 mM in the outflow to maximize the viability of the preparation on advice from Buchanan *et al.* (2001) (37; personal communication). The dry/wet ratio for the pancreas was similar to that seen for perfused liver tissue (31).

Physiological Spaces

This is, to our knowledge, the first published complete characterization of the physiological spaces of the perfused pancreas preparation using the multiple indicator dilution technique. The addition of RBC to the perfusate did not affect the distribution of the reference markers. This suggests that the hemodynamics of the system were similar with both RBC-free and RBC-containing perfusate. This is in accordance with results from other organ perfusion systems, in which addition of RBC to the perfusate did not affect the distribution of reference markers (16,31,38). The vascular and interstitial spaces are consistent with that reported in the pancreas previously; 0.18 (~0.15 ml/g tissue) for the fractional volume of vascular space (39), and 0.19 (30) and 0.207 ± 0.014 ml/g (40) for the interstitial space.

The reason for the recoveries of the reference markers and tolbutamide being less than 100% was most likely due to the leakage present in perfusions. Similar results have been seen in other heterogenous perfusion systems, with 86 ± 7% recovery of RBC and 71 ± 5/7% recovery of sucrose and water (41), and ~80% recovery of lidocaine (25) in hindlimb preparations and recoveries of 95 ± 11%, 85 ± 4% and 78 ± 7% for RBC, water and sucrose in the rat head (42).

A high CV^2 for all reference markers is similar to what has been reported in other heterogeneous organs such as the rat head (16). Whilst a high CV^2 may arise from low perfusate flow to certain areas including in the extreme, stagnation or trapping, the high CV^2 here is likely to reflect the heterogeneity in organs due to the presence of some duodenum in the pancreas perfusion. The liver, with a relatively homogeneous vascular system, has a lower CV^2 value (43).

Higher estimates of water volume in a perfused preparation than observed from wet weight ratios are due in part to the effects of capillary pressure and lack of lymph flow. The significant difference in the value between the parametric and non-parametric determination of the distribution of water appears to be a result of an overestimation by the parametric model. The larger volume for the parametric model is probably due, in part, to the assumption of an inverse Gaussian tail, leading to an over extrapolation of the tail section of the outflow curve.

Although there is over estimation with the model, the assumption that there is barrier-limited diffusion across the pancreas is suggested by the fact that the data was fitted using this model, with an estimated k_{in} that did not approach infinity. Furthermore, the simple space distribution model could not fit the data. Previous work has shown that there is a permeability limitation for water crossing the hepatocyte membrane in the liver (24). Hung *et al.* showed there was a direct relationship between fibrosis index and the hepatocyte PS product for water in disease models. The PS product of the control animals displayed evidence for the permeability limitation for water in the normal liver. The barrier-limited plus space-distributed model has been used extensively to estimate the water volume in liver perfusions (24,44–48).

Tolbutamide Distribution

The unbound distribution of tolbutamide is similar to water, suggesting that its main distribution sites are in the aqueous spaces of the perfusion preparation. The estimations of V_d, CL_{Bt} and $V_{t,u}$, suggest that tolbutamide's distribution in the preparation can be simply accounted to the physiological spaces and the relative binding between the perfusate and tissue. The PS value for tolbutamide was similar to water suggesting that the lipophilicity of tolbutamide (log *P* of 2.3 (49)) had promoted its partitioning into the cellular membrane but was offset by its size (270.35 MW).

The K_p values obtained in this study were comparable with *in vivo* data (based on steady state distribution) (50), when adjusted for the reported differences in the fraction unbound of tolbutamide ($f_{up}^{\text{present study}} = 0.36 \pm 0.01$; $f_{up}^{\text{invivo}} = 0.236$). There is always a concern that homogenisation of the tissue will change the binding for drugs by disrupting membranes, etc. However this does not appear to be the case in the present study, with the similarity in the estimated and experimental values of f_{ut} suggesting that homogenisation of tissue did not greatly affect binding.

In summary, a viable model to study pharmacokinetics in the perfused pancreas has been established; in which the physiological spaces of the preparation have been characterised using both parametric and nonparametric methods and the rate and extent of tolbutamide distribution has been described.

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