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Review

Application of Microdialysis in Pharmacokinetic Studies

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The objective of this review is to survey the recent literature regarding the various applications of microdialysis in pharmacokinetics. Microdialysis is a relatively new technique for sampling tissue extracellular fluid that is gaining popularity in pharmacokinetic and pharmacodynamic studies, both in experimental animals and humans. The first part of this review discusses various aspects of the technique with regard to its use in pharmacokinetic studies, such as: quantitation of the microdialysis probe relative recovery, interfacing the sampling technique with analytical instrumentation, and consideration of repeated procedures using the microdialysis probe. The remainder of the review is devoted to a survey of the recent literature concerning pharmacokinetic studies that apply the microdialysis sampling technique. While the majority of the pharmacokinetic studies that have utilized microdialysis have been done in the central nervous system, a growing number of applications are being found in a variety of peripheral tissue types, e.g. skin, muscle, adipose, eye, lung, liver, and blood, and these are considered as well. Given the rising interest in this technique, and the ongoing attempts to adapt it to pharmacokinetic studies, it is clear that microdialysis sampling will have an important place in studying drug disposition and metabolism.

KEY WORDS: microdialysis sampling; pharmacokinetics; drug distribution; probe recovery; blood-brain barrier; extracellular fluid.

INTRODUCTION

The technique of *in vivo* microdialysis has become one of the major research tools in experimental neurophysiology and neurochemistry. The large body of work published over the past three decades in this field underscores the importance of microdialysis, primarily in providing needed information concerning neurotransmitter release, uptake and metabolism.

Recently, *in vivo* microdialysis has found important applications in the field of pharmacokinetics, especially in the area of drug distribution and metabolism. This has been made possible by refinements and improvements in the quantitative aspects of the methodology, primarily those which characterize microdialytic recovery.

Principle of Microdialysis

Microdialysis involves perfusion of a microdialysis probe implanted in tissue (e.g., brain or peripheral tissues) under nonequilibrium conditions, i.e., the effluent concentration of analyte is less than that surrounding the probe membrane (see Figure 1). Therefore, dialysate concentrations of the solute or analyte of interest (Ces, the concentration of the solute in the effluent) are a fraction of the concentration in the extracellular space (Cos, the concentration of the solute outside of the probe in the extracellular fluid (ECF)). Knowledge of the fractional recovery (the ratio of the dialysate concentration to that in the extracellular fluid surrounding the probe membrane, Ces/Cos) of the solute or analyte is a prerequisite for calculating tissue extracellular concentrations of the analyte.

During perfusion of a dialysis probe, the driving force for solute movement is diffusion along the concentration gradient between two regions which are separated by a semi-permeable membrane. *In vivo*, these regions or spaces represent the tissue extracellular fluid (ECF) and the perfusing solution inside the microdialysis probe. Diffusion occurs through a polymeric membrane which encloses the tip of the dialysis probe. Endogenous compounds (e.g., hormones, neurotransmitters) and exogenous compounds (drugs and their metabolites) diffuse in, whereas compounds which have been added to the perfusate (e.g., the perfusate inflow concentration of a "calibrator" is represented by Cic in Figure 1), diffuse out from the perfusion solution. The technique is therefore used not only to continuously monitor the extracellular fluid concentration of analytes, but may also be used to deliver drugs to a specific tissue region. In essence, the principle of the microdialysis technique is to create an "artificial blood vessel" where diffusion of compounds will flow in the direction of lowest concentration.

There are basically three types of probes have been used in microdialysis: the loop probe, the concentric probe, and the linear probe. Although all three probe types have been used widely in neurochemistry and in experiments which have focused on endogenous compounds, much of the recent work in the field of pharmacokinetics has made use of the concentric probe design (see Figure 2). A cylindrical piece of dialysis tubing, which serves as the dialysis membrane, is sealed with

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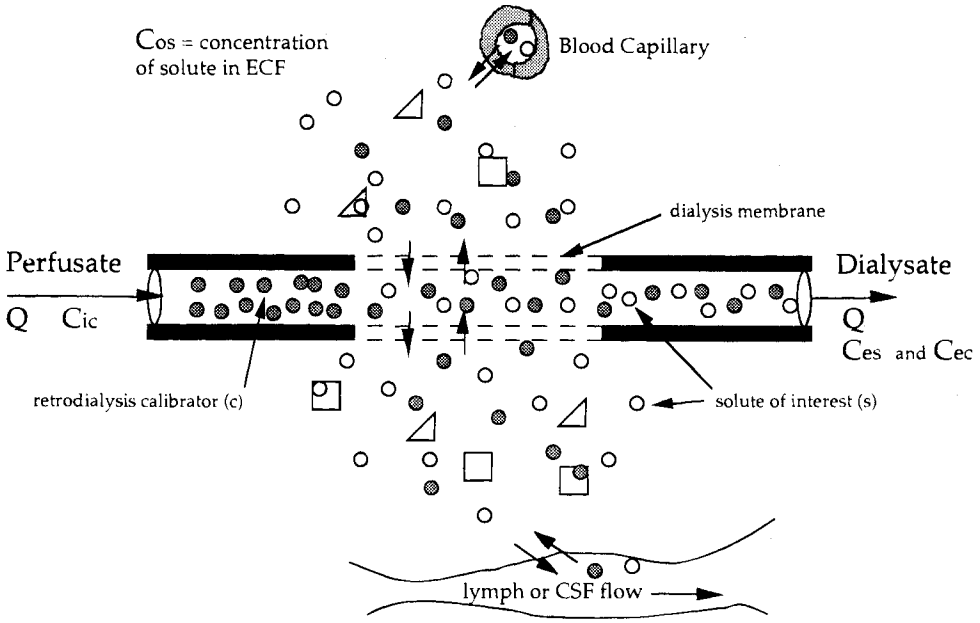

Fig. 1. Microenvironment within and surrounding the microdialysis probe *in vivo* (not drawn to scale). The solid and dased line segments schematically represent the nonpermeable probe wall and semipermeable membrane, respectively. Open and closed circles represent the molecules of the solute of interest and a retrodialysis calibrator, respectively. Squares and triangles represent macromolecules which may bind solute and/or calibrator, but which are not recovered by the dialysis process. Arrows indicate direction of mass transport. Abbreviations are as defined in the text.

an adhesive at one end and the other end is fixed to the lip of the outer shaft. A thin inner cannula, usually made of metal or fused silica, extends through the outer shaft and dialysis membrane, almost to its sealed tip. Perfusate solution enters the proximal end of the inner cannula and flows distally all the way to the end of the membrane where it is sealed. Every attempt is made to minimize the dead volume of the probe (the volume contained from the membrane to the outlet) to reduce the lag time associated with dialysate measurements in relation to the concentration in the ECF. The dialysis membrane material should be biocompatible and inert. The average pore size should be large enough to allow free diffusion of solute molecules, but small enough to restrict the passage of proteins and other macromolecules. Several different types semipermeable membranes have been employed, including: polycarbonate-ether, regenerated cellulose, and polyacrylonitrile (PAN) (1). Recently, a polyether sulfone (PES) membrane has been introduced with a greater molecular weight cutoff (100,000 daltons) than most other membranes (ca. 5,000 to 30,000 dalton MWCO). This material may make the microdialysis sampling of larger molecules feasible, opening new areas of application.

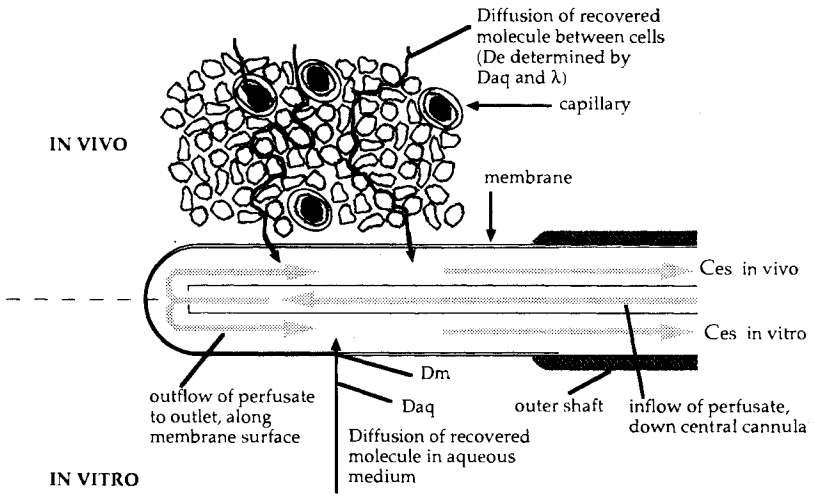

Fig. 2. Schematic representation of solute recovery *in vivo* and *in vitro* (not drawn to scale). See text for definition of terms.

Areas of Application in Pharmacokinetics

Microdialysis can be performed in the tissues of restrained or freely moving animals, or in animals which are maintained under anesthesia. Studies conducted in freely moving animals may allow for simultaneous assessment of pharmacodynamic response, and investigation of relationships between tissue concentrations of drug (and metabolite) and response. Clearly, such studies raise the possibility of identifying location of receptors, and comparing relative potencies of members of a homologous series.

The technique continuously monitors (for periods of hours or even days) the extracellular fluid of a specific tissue, as distinct from the whole tissue samples collected by biopsy, or after sacrificing the animal. On the other hand, measured concentrations of drug in whole tissue homogenates represent a weighted average of the extra- and intracellular concentrations, and they also may reflect amounts of analyte which are contributed from blood entrapped in the tissue.

It is important to recognize that microdialysis sampling is a continuous process that results in drug concentration vs. time data that are a reflection of the mean concentration at the sampling site (when adjusted for recovery) over a discrete time interval. This contrasts with traditional pharmacokinetic sampling in blood or tissue which yields drug concentrations at discrete time points. This distinction results in different methods to calculate the area under the concentration vs. time curve (2–4). The drug area under the curve (AUC) for the microdialysis-sampled tissues are calculated as the sum of the products of the measured concentration in an interval and the time of the collection interval, with addition of the residual area to obtain the AUC to infinite time. This calculation is made possible because the concentration measured in the dialysate is essentially the average concentration over the collection interval and as such, an integrated response value (the time-averaged concentration). As is true with any pharmacokinetic study which uses traditional discrete-point sampling, the microdialysis sampling frequency, i.e., the temporal resolution of the concentration data, may affect various pharmacokinetic parameters, such as time to maximum concentration, t_{max} (5). The sampling frequency is primarily dependent on the speed and sensitivity of the available analytical methods.

Microdialysis permits the investigator to examine intra-animal variability in tissue measurements as a function of time. This avoids the problems associated with interanimal variability which often complicates pharmacokinetic interpretation of studies involving collection of data from a population of animals. It also reduces the total number of animals required in experimental work.

Microdialysis can be performed in almost every organ or tissue of the body. In the study of solid tissues, probes are usually implanted at specific coordinates, using a stereotaxic apparatus and the permanent placement of guide cannulas. Indeed it is possible to implant more than one probe in the brain of the freely moving rat and simultaneously monitor drug concentrations at multiple sites. Although most pharmacokinetic studies to date have focused on distribution into the brain (e.g., 6–8), investigators are now examining dialysis of muscle (9–11), systemic blood (12), liver (13), heart (14), subcutaneous tissue (15,16), and in the assessment of plasma protein binding (17,18). Studies of drug distribution, metabolism, and absorption utilizing microdialysis have been conducted primarily in the rat, but other species, including man, have been the subject of these investigations as well.

Recovery/Calibration Issues

The rapid spread of interest in microdialysis in the study of endogenous compounds such as neurotransmitters occurred in part because these studies often related changes in the levels of these compounds to their baseline values. Under such conditions, recovery *in vivo* was not a critical issue. However, in studies of the distribution and metabolism of exogenous compounds (i.e., pharmacokinetic studies), there is no baseline concentration of the exogenous substance (i.e., the drug and/or its metabolites) to which subsequent measurements can be related. The interpretation of data derived from pharmacokinetic studies utilizing microdialysis therefore requires an understanding of the qualitative and quantitative processes involved. A number of publications have addressed these points (7,19–27). A comprehensive review of the quantitative aspects of this technique has recently appeared (28), and an excellent volume which provides a detailed analysis of the field has also been published (29).

Since microdialysis operates under nonequilibrium conditions, the concentration of analyte in the dialysate is less than that in the extracellular fluid surrounding the probe. The ratio between these concentrations is defined as relative recovery. The higher the flow rate, the further removed will be the effluent concentrations from an equilibrium with the unbound concentrations in the extracellular fluid, and the more dilute will be the dialysate samples collected. Thus, relative recovery is decreased as perfusate flow rate is increased. This is shown in Figure 3, where the recovery of the antiviral nucleoside AZT (and the loss of an analog with similar properties, AZdU, to the region outside the probe membrane) is shown to be an exponential function of dialysate flow rate (30).

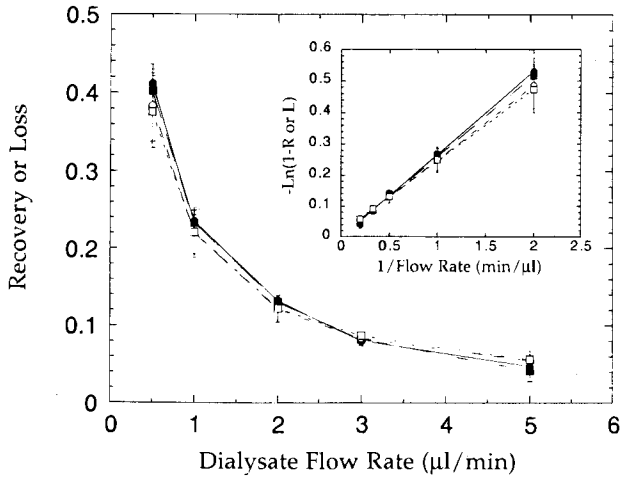

Fig. 3. Effect of flow rate on recovery of AZT and loss of AZdU during microdialysis and retrodialysis *in vitro*. Inset: Linear relationship between the transformation of recovery (R) or loss (L) and dialysate flow rate during microdialysis and retrodialysis *in vitro*. AZT: recovery (□); loss (■). AZdU: recovery (○); loss (●). Mean ± SD for 4 probes are shown (some data overlap).

In an effort to characterize the concentration of solutes in extracellular fluids from a knowledge of dialysate concentrations, Jacobson *et al.* (31) described an approach utilizing a steady-state mass transfer relationship which considers changes in dialysate concentration at different flow rates. This method is also called the flow-rate or stop-flow method. Lonnroth *et al.* (15) developed an elegant method where recovery *in vivo* is estimated from dialysate concentrations when a wide range of concentrations of the solute of interest are perfused, while maintaining the extracellular concentration *in vivo* at steady state. This is referred to as the concentration difference method or zero-net flux method (ZNF). The zero-net flux method is a straight forward approach to calibrating probes *in vivo* because it makes no assumption regarding mass transfer within the tissue. Under steady state conditions, different steady state concentrations of the compound of interest are perfused through the probe (Ci) and the steady state concentrations in the effluent dialysate (Ce) are measured. When the concentration difference (Ce-Ci) is plotted as a function of Ci the line of best fit will intersect the abscissa at the value of Ci which equals the concentration in the extracellular fluid (the point of zero-net-flux). If a linear relationship and true steady state conditions exist, the negative slope of the line provides the microdialysis probe recovery of the compound (see Figure 4 and discussion below).

The need for characterizing recovery *in vivo*, rather than relying upon values of recovery determined *in vitro* stemmed from observations by a number of investigators that differences in diffusion coefficients *in vitro* and *in vivo* were extremely large. The reasons for these differences are explained by comparing the environments in which a hypothetical molecule exists *in vitro* and *in vivo*. This is shown in Figure 2, which examines differences in recovery of solutes under *in vitro* and *in vivo* conditions. In vitro, flux is limited by the diffusion coefficients

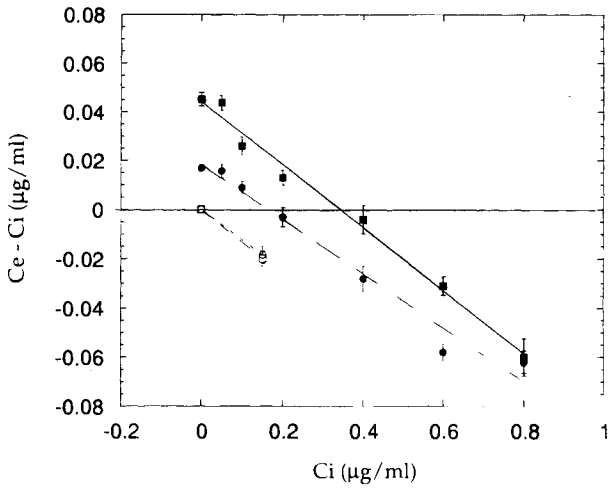

Fig. 4. Microdialysis of the ventricle (circles) and thalamus (squares) in one rabbit using zero-net flux method (ZNF; solid symbols) and simultaneous retrodialysis (RD; open symbols) at a flow rate of 1 µl/min. Rabbit received a constant-rate IV infusion of AZT for at least 6 hr prior to ZNF and RD calibration. Axes refer to solute and calibrator concentrations for ZNF and RD methods, respectively. Ordinate values are mean ± SD for ZNF data (closed symbols, n = 3 or 4 at each concentration) and for RD data (open symbols, n = 25). The ZNF data for ventricle and thalamus were fitted by simple linear regression. Slopes are estimates of recovery of AZT (ZNF) and loss of AZdU (RD).

in aqueous solution (Daq) and in the membrane (Dm). However, diffusion of molecules recovered by the probe *in vivo* is constrained by a volume fraction which represents the ratio of extracellular space to tissue volume (ECF represents approximately 15 to 20% of the tissue volume). The tortuous pathway taken by recovered solute molecules results in an effective increase in path length, characterized by λ. Furthermore, the microviscosity of the extracellular fluid (ECF) is greater than that of water due the presence of structural macromolecules. Association or interaction of solute molecules with these macromolecules also reduces the effective transit rate, further reducing the diffusivity of solute through tissue ECF. It is likely that these factors contribute measurably to the observed differences in recovery of solute determined *in vitro* and *in vivo*.

Through an examination of fundamental transport and physiological parameters, Lindefors and colleagues (20,21) developed a detailed mathematical model of microdialysis that recognizes the importance of tortuosity as well as diffusion and convection phenomena in the tissue, probe membrane, and probed medium. In addition, Bungay *et al.* (23) presented a steady-state diffusion model that considered the effects of microvasculature transport, metabolism, and intra/extracellular space exchange. Recently, Morrison *et al.* (7) extended the model of Bungay et al. and described transient probe response when metabolism or microvasculature transport play major roles. These models have greatly advanced our understanding of factors governing spatial and time-dependent solute distribution.

Stahle *et al.* (32) recently compared the methods of Jacobson *et al.* (31) and the zero-net-flux method introduced by Lonnroth *et al.* (15) for calibrating probe recovery. He concluded that both gave satisfactory results, with the zero-net flux method of Lonnroth et al. possibly yielding more accurate predictions.

Because the zero-net-flux method requires that the study subject be examined under steady-state conditions prior to the actual experiment, the total study time is extended. In view of observed time-dependent changes in relative recovery (25,33), it is possible that recovery calculated using a zero-net flux approach may not be applicable during the subsequent experimental period. Retrodialysis, which allows for continuous assessment of recovery *in vivo* during the study period (8,34) may avoid this potential problem. Here, retrodialysis is defined as the diffusive loss of molecules from the dialysis perfusate into the environment surrounding the probe, under sink conditions. To employ this technique, a calibrator whose dialysance or permeability-area product (PeA) is similar to that of the compound of interest is introduced into the perfusate (in Figure 1, Cic is the calibrator concentration in the entering perfusate) and its relative loss is measured (in Figure 1, Cec is the calibrator concentration in the dialysate effluent). The relative loss of an ideal calibrator is identical to the recovery of the solute during normal microdialysis. The recovery and loss of the solute and its retrodialysis calibrator are functions of their effective permeabilities, which are reciprocally related to their resistance to diffusion (30).

The principle underlying the calibration of dialysis recovery by the method of zero-net flux is further illustrated by the following discussion. The method requires that the concentration of solute outside the probe is fixed, e.g., at steady state. The direction of the gradient across the dialysis membrane

depends upon how the concentration in the perfusion medium compares with that in the fluid surrounding the membrane. By varying the concentration in the perfusion medium over a sufficiently large range, the magnitude and direction of the gradient is altered. Rs and Ls represent the relative recovery of the solute into dialysate and its relative loss from perfusate. Using other symbols defined above, it is seen that:

$$Ces = Rs \cdot Cos + (1 - Ls) \cdot Cis \quad (1)$$

It follows that:

$$Ces - Cis = Rs \cdot Cos - Ls \cdot Cis \quad (2)$$

The concentration in the medium surrounding the probe, Cos, at which the net flux across the membrane is zero, can be estimated from equation 2, by linear regression of the concentration difference (Ces-Cis) on Cis, as the intercept on the X-axis. The slope is −Ls, where Ls is equal to Rs, the relative recovery of the solute. When the net flux is zero (Ces − Cis = 0), the following is obtained:

$$Rs \cdot Cos - Ls \cdot Cis = 0 \quad (3)$$

Thus, Cos equals Cis when Rs equals Ls. Since this analysis depends upon the linear relationship expressed by Eq. 2, it assumes that recovery (the negative slope of the line) is independent of solute concentration. This requires that the diffusivity of the solute molecule in the recovery media (membrane, tissue ECF, etc.) is independent of concentration.

Retrodialysis (RD) is a special case of a net flux analysis, where an analog of the solute of interest, referred to as a calibrator, is added to the perfusate, instead of the solute itself. The concentration of the calibrator, whose diffusivity is similar to that of the solute of interest, is zero in the region outside the probe membrane. Thus, its net flux is always negative, i.e., it is lost from the perfusate. Where the loss of the calibrator, Lc, is shown to be equal to Rs, the use of the RD calibrator is validated. Here, Lc is the negative slope of a ZNF plot, as shown in Figure 4.

An example of the simultaneous use of ZNF and RD to allow for a comparison of estimated tissue concentrations of azidothymidine (AZT) in rabbit brain has been reported (30). In this study, concentrations of AZT in ventricle and thalamus were maintained at steady state by constant-rate IV infusion of AZT (n = 4). Infusion was maintained for at least 6 hours before dialysis commenced. Dialysis probes were inserted into the ventricle and thalamus guide cannulas. To conduct the ZNF analysis, a series of solutions in which the AZdU concentration was fixed (0.15 μg/ml), and AZT concentrations varied (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml) were perfused in sequence through both probes. Three to four dialysis samples at steady state were assayed on-line by HPLC for AZT and AZdU concentrations. The AZT analysis yielded a probe recovery calculation through the construction of a ZNF plot, whereas AZdU analysis permitted simultaneous measurement of probe recovery by determining the loss of this calibrator. Blood samples were collected at the midpoint of each period from another ear vein catheter to allow for confirmation of steady state. CSF (30 μl) was sampled from the cisterna magna of one rabbit to permit comparison of AZT concentrations estimated by RD and ZNF with those measured directly.

Figure 4 illustrates representative results obtained using ZNF and RD in a rabbit with dialysis probes inserted in both the thalamus and ventricle. Good correlations between the concentration differences ($C_e - C_i$) and concentrations in perfusate (C_i) were obtained for thalamus ($r^2 = 0.97$) and ventricle CSF ($r^2 = 0.99$). Relative recoveries of AZT were estimated as the negative slope of the linear regression line (solid symbols). Tissue concentrations (C_{csf} and C_{ecf}) were calculated directly as the intercepts of the regression lines on the X-axis. The relative loss of AZdU in thalamus and ventricle probes during the entire experiment was also obtained simultaneously by retrodialysis, and is shown as the slope of the line segment for the AZdU data (open symbols) in Figure 4.

Simple linear regression of the average loss of AZdU during RD upon the relative recovery of AZT estimated using ZNF (Figure 5) yielded a linear relationship with a slope of 0.97 and intercept of 0.008 (line of identity is shown rather than regression line) and good correlation ($r^2 = 0.94$). It can be observed from the relative position of the open symbols (representing results *in vitro*) that recoveries *in vivo* are significantly less than those determined *in vitro*. Thus, calibration of probe recovery was comparable by both methods, both *in vitro* and *in vivo* (30).

INTERFACING WITH ANALYTICAL INSTRUMENTATION

Microdialysis sampling has proven to be a useful technique for a wide range of pharmacokinetic and drug metabolism studies, however, an investigator must be aware of several special analytical considerations to successfully apply this technique. Microdialysis is a continuous sampling method which yields small sample volumes due to the low perfusate flow rates utilized to enhance relative recovery. Microdialysis perfusates can be collected over discrete sampling intervals and then subjected to analysis. The temporal resolution of these sampling intervals depends on the sensitivity of the analytical method employed, and the time needed to perform the analysis. Several analytical methods have been employed to measure compounds

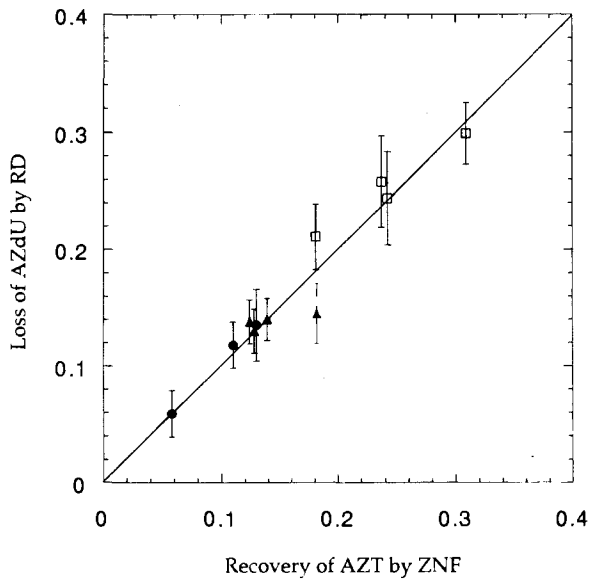

Fig. 5. Agreement between retrodialysis loss of AZdU and the zero-net flux recovery of AZT, *in vivo* (thalamus, ● ; ventricle, ▲) and *in vitro* (□). The line of identity is shown.

of interest in microdialysates. Representative examples include liquid chromatography (35,36), mass spectroscopy (37), capillary electrophoresis (38), and immunoassays (39).

In spite of volume limitations, microdialysis samples have some advantages over traditional biomatrix samples. The dialysis membrane excludes protein from the aqueous sample, therefore no sample preparation is necessary and enzymatic degradation is not a concern. Thus, the direct on-line coupling of an analytical technique to microdialysis sampling is possible, and has been utilized for HPLC, capillary electrophoresis, and mass spectroscopy methods. On-line systems, where the microdialysate is introduced directly into the analytical system, reduce the problems associated with sample transfer and evaporation of the small volume microdialysates. In addition, on-line coupling of the analytical instrumentation to the sample flow allows for the analysis of samples in near real time, providing rapid feedback of data.

On-line analysis typically involves coupling the microdialysis system to a microbore HPLC system. Microbore chromatography increases the mass sensitivity of the analytical system, and speeds the time of analysis, both important considerations for on-line microdialysis sampling (35,40). Chen and Lunte (41) report *in vitro* experiments where caffeine, theobromine, and paraxanthine could be resolved under 1 minute, and acetaminophen and its two main metabolites, the glucuronide and sulfate conjugates, could be resolved in 30 seconds. *In vivo* pharmacokinetic experiments were done following intravenous bolus dosing of acetaminophen and caffeine, with a temporal resolution of the plasma concentrations under 1 minute.

Various detectors have been used with the HPLC analysis of microdialysates depending on the analyte. Electrochemical detection has been popular in the analysis of biogenic amines such as dopamine, serotonin and norepinephrine (42–44). Simultaneous measurement of monoamines, metabolites, and 0-phthaladehyde (OPA)-derivatized amino acids in brain microdialysis perfusates has been achieved using electrochemical detection and column switching (45). Fluorescence (e.g., 46,47) and ultraviolet (e.g., 48,49) detection has been used for pharmacokinetic studies that used microdialysis sampling.

Capillary electrophoresis (CE), with its small injection volume requirements, may be particularly suitable to improve time resolution of microdialysis sampling, especially when improved detection techniques are employed. Tellez *et al.* (50) used off-line CE to determine phenobarbital concentrations in blood and brain dialysates. The results of that study demonstrated that CE is a feasible means of analyzing microdialysates for pharmacokinetic studies. A specialized interface between the perfusate flow and the injection stream of the CE system has been developed by Hogan *et al.* (51), making on-line sampling possible for CE. Using this specialized interface with high-speed micellar electrokinetic separation (MEKC), resolution of the investigational antineoplastic SR 4233 and its main metabolite was achieved in less than 60 seconds, allowing the on-line system to achieve a temporal resolution of 90 seconds for determining the pharmacokinetics of SR 4233 *in vivo* (51). Zhou *et al.* (38) describes a "separation-based biosensor" which consists of an on-line microdialysis sampling CE system that uses laser-induced fluorescence to enhance detection. Detection limits for the amino acid neurotransmitters, glutamate and aspartate, were 0.1 micromolar, with separation achieved in 70 seconds.

Microdialysis techniques have been successfully interfaced with mass spectroscopy (MS). Fast-atom bombardment MS has been used to follow the pharmacokinetics of penicillin G in the rat (37). Thermospray tandem MS has been employed to monitor the experimental dopamine uptake blocker, GBR-12909 in brain interstitial fluid (52). An automated HPLC/MS system coupled on-line with microdialysis sampling has been used for the *in vivo* analysis of contrast agents (53). On-line coupling of *in vivo* microdialysis with tandem MS was utilized to determine the pharmacokinetics of tris(2-chloroethyl) phosphate in the rat (54), and compared with conventional sampling methods (55). Gamma-aminobutyric acid (GABA) levels in the rat brain have been measured using the coupling of microdialysis sampling with capillary electrophoresis/mass spectroscopy without requiring any sample pretreatment (56).

Many recent advances have occurred in interfacing analytical instrumentation with microdialysis sampling, particularly in developing on-line techniques. Microdialysis sampling is becoming more popular in pharmacokinetics and drug metabolism, and with concurrent improvements in specialized analytical technology to address the specific needs such as sensitivity, small volume, and continuous collection issues, the microdialysis technique will become more applicable to a wide range of pharmacokinetic problems.

REPEATED PROCEDURES

Microdialysis sampling has been extensively utilized in neuroscience for short term sampling in the central nervous system for acute experiments in anaesthetized animals and chronic experiments where the animals have been allowed to recover from anesthesia. These experiments usually involve a between-subjects study design (57). One disadvantage with this design is that the same animal cannot be evaluated repeatedly over time. A more powerful study design in many pharmacokinetic and pharmacodynamic applications would be a within-subjects design, in which each animal is tested with more than one experimental condition, allowing each animal to serve as its own control. This design circumvents variation due to individual differences and would make it possible, among other applications, to: 1) determine pharmacokinetics following acute and chronic drug administration within the same animal, 2) do dose-ranging studies to examine the dose dependence of pharmacokinetic parameters within the same animal, 3) investigate pharmacokinetic drug interactions using a crossover design, and 4) significantly reduce the number of animals necessary to achieve experimental goals.

Microdialysis sampling coupled with a crossover study design has been used to study the effect of transport inhibitors on the distribution of compounds into the CNS (48,58,59). The effect of probenecid on the distribution of zidovudine in the rabbit brain has been studied using microdialysis with a crossover design that included a repeated control to examine the possibility of a period effect (48). Wang *et al.* (59) have studied the effect of cyclosporin A on the distribution of rhodamine-123 into the rat brain using a two way crossover, balanced for treatment order. Each of these studies saw no treatment order effect in the CNS distribution of these compounds and the relative recovery of the probes, as determined by the retrodialysis method, were stable with time. The feasibility of performing repeated microdialysis experiments within individual

rats in studying the CNS distribution of two model drugs, acetaminophen and atenolol, representing a moderately lipophilic and hydrophilic drug, has been examined by de Lange *et al.* (60). Repeated experiments with isotonic perfusate solutions (24, 48, and 72 h post-surgery) resulted in AUC brain ECF values with the ratio of 100:103:76% for acetaminophen and 100:103:98% for atenolol. It was concluded that intracerebral microdialysis can be used to study the pharmacokinetics of drugs in the brain, provided that experiments are conducted under carefully controlled conditions (60).

The reliability of repeated microdialysis has been questioned because of lack of sufficient information about possible interfering factors, such as tissue changes (61). Generally, the reliability of longitudinal studies using microdialysis sampling would have to be verified for each experimental situation, given the possible differences between tissue types (e.g., brain, blood, muscle) in the local reaction to probe. One important consideration would be the stability of the relative recovery of the probe over time. The time course of any changes in recovery could be monitored using the retrodialysis method or by monitoring the dialysate concentration of a substance which is known to be at a constant concentration at the sampling site throughout the course of the experiment (such as an endogenous marker). Monitoring the relative recovery over time can also give an indication about the condition of the tissue surrounding the probe site, if the tissue condition is important in determining recovery related factors such as diffusivity, metabolism, and transport.

The effect of probe implantation on the surrounding tissue has been examined in the brain, the tissue where the technique has been most frequently employed. It has been shown that minimal tissue changes occur within 1 to 2 days following implantation, with astrocyte hypertrophy present at 3 days and connective tissue changes after 2 weeks (62). Eicosanoid levels in the striatum have been measured following the introduction of a microdialysis probe into the tissue (63). Formation of PGD_2, $PGF_{2\alpha}$, and TxB_2 was highest in the first hour following introduction of the probe into the rat striatum, however, the level of PGE_2 was highest during the sixth hour of collection. Functional parameters such as regional cerebral blood flow and glucose phosphorylation have been studied and pronounced changes in these parameters occur during the first 2 hours after implantation reverting to near normal levels after 24 hours (64). Repeated probe insertions has been used to study the dopaminergic system in the striatum of rats (65). There was no significant difference in basal concentrations of dopamine between two test sessions separated by one week, however, the amphetamine-simulated dopamine release was greatly attenuated following the second probe insertion. These changes emphasize the need to evaluate repeated sampling designs for each study, depending on the pharmacokinetic and/or pharmacodynamic measurements required to meet the goals of the study.

A critical factor in the use of microdialysis sampling for the many pharmacokinetic studies of drug transport in the CNS is the integrity of the blood-brain barrier following probe implantation and the time course for the re-establishment of the barrier properties. This has been examined using radiolabelled solutes that are normally excluded from the brain extracellular fluid, such as ^{14}C-sucrose, ^{14}C-mannitol, ^{3}H-inulin, $Na^{99m}TcO_4$ and a non-radiolabelled marker, 4-trimethlyammonium antipyrine (66). In an early report, Tossman and Ungerstadt have shown after implantation of a probe in the brain the penetration of $Na^{99m}TcO_4$ (0.16%) through the BBB was minimal in comparison to $^{3}H_2O$ (52%) (67). A similar result for technetium oxide has been reported by Aamundstad et al. (68) with a transfer across the BBB of 0.11 ± 0.05% for 12 animals, which was increased to 1.89% in one animal that had a loose guide cannula, suggesting possible damage to the barrier. Terasaki et al. studied the damage due to a transcranial type probe using radiolabelled sucrose as the extracellular marker and found the average brain ECF to plasma ratios of ^{14}C-sucrose were 7% at one hour after implantation and 3.7% after 48 hours (69). Relative penetration ratios calculated from reported permeability-surface area products for sucrose in the brain capillary (70) range from 2.2 to 11%, which is in good agreement with the experimentally derived values from the microdialysis experiments (63). Fontaine *et al.* (71) measured the cortex ECF to plasma AUC ratios (corrected for in vivo probe recovery) for $^{3}H_2O$ and ^{14}C-mannitol 18 hours after probe placement in the freely-moving rat. Following intravenous injection of each marker, the relative penetration (AUCcortex/AUCplasma) of water was 103% (n = 2) and the penetration of mannitol was 9.3% (n = 6), indicating the chemical selectivity of the BBB was intact.

Other studies (72,73) have questioned the intactness of the BBB after probe placement, measuring increased albumin immunoreactivity, Evans blue tissue penetration and ^{51}Cr-EDTA transfer. Moreover, Westergren *et al.* (72) found no significant increases in the ^{3}H-inulin found in the dialysate following an intracarotid injection of protamine sulfate designed to enhance the permeability of the BBB. However, Morgan et al. used the microdialysis and classic tissue homogenate methods to examine the apparent brain uptake of two polar solutes, ^{3}H-sucrose and ^{14}C-urea (74). These authors state that the results from this study suggest that the blood-brain barrier remains compromised for some time after microdialysis probe insertion. It is clear that additional investigation is needed to clarify the issue of blood-brain barrier integrity following probe insertion, and how the traditional brain tissue homogenate data can be compared to microdialysis sampling of the brain extracellular fluid.

PHARMACOKINETIC STUDIES UTILIZING MICRODIALYSIS SAMPLING

Central Nervous System

Until recently, the use of microdialysis sampling for pharmacokinetic studies has seen its principal application in examining drug distribution and transport in the brain. This stems, to some extent, from the fact that the microdialysis technique was initially developed to study various aspects of neurochemistry, and also from the fact that the rigid design of the early microdialysis probes was suitable for intracerebral placement. Brain microdialysis has some advantages over other methods to measure brain distribution and transport of drugs, such as serial sampling in a single animal, excellent temporal and spatial resolution to measure unbound drug concentrations in the CNS, and high selectivity in determining the analyte of interest without interference from metabolites.

There have been several excellent publications (e.g., 7, 28, 75) regarding a variety of the quantitative aspects of microdialysis sampling in the brain to study drug distribution and transport, and the current review does not attempt to give a comprehensive discussion about these considerations. The intent of this section of the review is to give recent examples of a variety of applications of the microdialysis sampling technique in the field of CNS neuropharmacokinetics, therefore most of the following examples are listed by drug class.

Analgesics

Brain microdialysis has been applied to determine the distributional pharmacokinetics of analgesic agents to the brain, including analgesic peptides (76), acetaminophen (77), and opiates such as morphine and selected metabolites (68,78–81). Terasaki *et al.* examined the transport of a synthetic opioid octapeptide, E-2078, through the blood-brain barrier of anesthetized rats using a transverse intracerebral microdialysis probe (76). These authors found a significant transport of the peptide across the blood-brain barrier, however, quantitation of these results should be viewed with caution, since *in vitro* probe recoveries were used to estimate *in vivo* extracellular fluid concentrations. This issue was discussed in another microdialysis study designed to determine the pharmacokinetics of acetaminophen in blood and CSF after an intravenous bolus dose of 25 mg/kg to rats (77). *In vitro* recovery values were greater than *in vivo* values measured by retrodialysis for both blood and CSF probes, with acetaminophen recovery *in vivo* 50 to 60% less than *in vitro*. When correcting dialysate concentrations using recovery values determined *in vivo*, microdialysis derived pharmacokinetic parameters from this study were similar to results of other investigators that used traditional sampling methods for CSF and blood (77). Numerous studies have used microdialysis sampling to study morphine pharmacokinetics in the brain (68,78–81). Aasmundstad et al. have used microdialysis to sample morphine and morphine 6-glucuronide in striatal extracellular fluid following subcutaneous administration of morphine or metabolite to freely-moving rats (68). Their results indicate that the active metabolite of morphine, morphine 6-glucuronide, penetrates into the brain following systemic administration in rats.

Antibacterial and Antifungal Agents

Microdialysis sampling has been employed to examine the CNS distributional kinetics of some antibacterial and antifungal compounds. Mindermann *et al.* measured the penetration of rifampicin into the cerebral extracellular space of the anesthetized rat following a 100 mg/kg intraperitoneal dose using both the low flow and zero net flux methods to determine probe relative recoveries (82). Depending on the recovery method, the rifampicin concentrations in the extracellular fluid of the brain were 0.3 to 1% of the corresponding serum concentration, indicating a relatively poor penetration into the CNS. In another study, Granero et al. used microdialysis to examine the distribution of ceftriaxone and ceftazidime, cephalosporins commonly used to treat bacterial meningitis, in two regions of the brain (striatum and lateral ventricle) of the awake, freely moving rat (83). Antibiotic concentrations, measured at steady state, were calculated using *in vivo* recovery determinations by the extrapolation-to-zero flow rate method described by Jacobson *et al.* (31). This study found that the concentrations of both antibiotics in the CNS were significantly lower than the total concentrations in plasma (0.5 to 3%). The distribution of a novel triazole antifungal agent to the brain has been studied using *in vivo* microdialysis in a freely-moving rat model following intravenous bolus doses of 10 and 20 mg/kg (84). In this crossover study the fluconazole concentration in the cortical extracellular fluid was measured using the retrodialysis technique to determine *in vivo* probe recovery. Results indicate that fluconazole rapidly reaches a distributional equilibrium between the brain extracellular fluid and plasma, and the distribution to the brain is substantial (brain/plasma AUC ratio = 60%) and not dependent on dose over a two-fold range (84). These studies indicate that using microdialysis can be a useful technique to determine the penetration of antibacterial and antifungal drugs into the CNS, which may be crucial in evaluating the potential therapeutic efficacy of these compounds in treating cerebral infections.

Nucleoside Antiviral Agents

Additional anti-infective agents whose pharmacokinetics have been studied using microdialysis include the nucleoside antivirals. There has been considerable interest in characterizing the brain distribution of antiviral agents, given the important neurologic sequelae in AIDS. Wong et al. studied the distributional characteristics of zidovudine across the blood-brain barrier and blood-CSF barrier in the conscious rabbit using microdialysis sampling that employed the retrodialysis technique to determine in vivo relative recovery of probes in the thalamus and lateral ventricle (8). This study was a crossover dose-ranging study where rabbits received 5, 10, 20 and 30 mg/kg zidovudine as an intravenous bolus dose. A comparison of the terminal half-lives and AUC ratios indicated that there were no dose-related differences in zidovudine distribution or elimination kinetics. Zidovudine rapidly equilibrated between the thalamus extracellular fluid (ECF) and plasma, and the thalamus ECF/plasma AUC ratio was 0.09. The zidovudine CSF/plasma AUC ratio was 0.18, which is consistent with that observed (0.15) following direct CSF sampling from the cisterna magna (85), suggesting that the recovery of zidovudine estimated by retrodialysis is reliable. The calibration of the zidovudine microdialysis recovery using retrodialysis was validated *in vivo* when compared to the *in vivo* zero-net flux method of recovery determination (30). Wang and Sawchuk have further investigated the distribution of zidovudine between plasma, brain ECF, and CSF following intravenous and intracerebroventricular administration in a crossover study in unanesthetized rabbits (86). The transport processes were modeled by pharmacokinetic compartmental analysis, and the results suggest that the brain-to-blood efflux clearance was five fold greater than the blood-to-brain influx clearance and the CSF-to-blood clearance was three times greater than the blood-to-CSF clearance (86).

The effect of the anionic transport system inhibitor, probenecid, on the brain distribution of zidovudine has been studied using *in vivo* microdialysis in both the rabbit (48,86) and the rat model (27). Wong et al. examined the effect of probenecid on the distributional transport of zidovudine between plasma, CSF and brain extracellular fluid in a crossover design in rabbits that were instrumented with microdialysis probes in the lateral ventricle and the thalamus (48). Probenecid decreased the total

body clearance of zidovudine, but also decreased the zidovudine clearance from the CSF and thalamus ECF, prolonging the halflives of elimination from the brain. Moreover, probenecid treatment increased the zidovudine AUC in the ventricular CSF 3- to 5- fold and the AUC in the thalamic ECF by 5- to 6-fold, whereas the plasma AUC increased only 2-fold. These results provide evidence that zidovudine is transported from the CSF to the blood and the thalamic ECF to the blood by a probenecid-sensitive transport system. Using the CSF, ECF, and plasma concentration-time data as input, a pharmacokinetic model describing the competitive inhibition of efflux clearance estimates that approximately 75% of the clearance out of the CSF and brain ECF is subject to inhibition by probenecid (48). Dykstra et al. have uniquely combined the methods of microdialysis and quantitative autoradiography to examine the effect probenecid has on the transport of zidovudine in the brain extracellular fluid in the rat (27). Zidovudine concentration-distance profiles emanating from an acutely implanted microdialysis probe were measured using quantitative autoradiography. Coadministration of probenecid led to a substantial increase in the diffusion distance from the probe, suggesting that zidovudine is actively transported out of the brain ECF to the blood and that this mechanism is responsible for the limited penetration of zidovudine into the brain, in spite of its lipid solubility (27).

In another nucleoside study, Ljungdahl-Stahle et al. examined the brain and muscle penetration of zidovudine and 3′-fluoro-3′-deoxythymidine (FLT) in cynomolgus monkeys using microdialysis sampling, and found that the concentration of zidovudine and FLT in the brain was approximately one-third of that in the blood (87). Stahle and Oberg have studied the pharmacokinetics and distribution across the blood-brain barrier of two acyclic guanosine analogs (acyclovir and a quanine analog, H2G) using intracerebral microdialysis in anesthetized rats (88). Their study calibrated *in vivo* recovery by estimating the loss of each analog from the probe perfusate to the brain prior to subcutaneous injection of 25 mg/kg of the gaunosine analogs. The concentrations attained in the brain were slightly lower for H2G than for acyclovir (88).

Drug Distribution to Brain Tumor

Intracerebral microdialysis has been used as a tool to investigate drug distribution into brain tumors. De Lange *et al.* (89) determined the methotrexate concentration-time profiles in brain cortical dialysate and plasma following a 75 mg/kg intravenous dose. The R-6 rhabdomyosarcoma was implanted in the left cortex, and using a transverse microdialysis probe cortical ECF concentrations of methotrexate were measured in both normal and tumor-bearing brain. Methotrexate distribution to cortical brain at the tumor site was enhanced 2.5 fold over normal brain, and tumor tissue was determined to be the most important parameter in increasing local methotrexate concentration after tumor implantation. The authors concluded that this study indicated that intracerebral microdialysis is suitable to study the pharmacokinetics of anticancer drugs in brain tumor (89). Recently, other investigators have used microdialysis sampling to examine the free methotrexate concentrations in the brain extracellular fluid of normal and RG-2 glioma bearing rats following a 50 mg/kg intra-arterial dose of methotrexate (90). These authors also found a significant increase in the penetration of methotrexate into the tumor tissue, and a hybrid physiologically-based pharmacokinetic model was used to characterize the mechanisms that may be responsible for the high methotrexate tumor concentrations (90). This same group has examined the uptake of temozolomide (a DNA-alkylating agent with brain tumor activity) in a subcutaneous model of C6-glioma in the rat using microdialysis sampling of the tumor extracellular fluid (91).

Related to the microdialysis study of drug distribution to brain tumor tissue are the studies that utilize microdialysis to investigate to functional activity of the p-glycoprotein drug efflux pump (MDR, multi-drug resistance pump) in the blood-brain barrier. Sakata et al. measured the distribution of cyclosporin A to the brain of rats in the presence and absence of quinidine, a known p-glycoprotein inhibitor (92). They used a transverse microdialysis probe to locally administer the quinidine to the hippocampus. In the presence of the quinidine delivery via the microdialysis probe, the steady-state brain to blood efflux rate constant for cyclosporin A decreased to 5% of control, indicating inhibition of the cyclosporin brain to blood transport (92). In another study examining p-glycoprotein function in the blood brain barrier, Wang *et al.* measured the cortical ECF concentration of rhodamine-123, a high affinity p-glycoprotein substrate, with and without coadministration of cyclosporin A in crossover design using microdialysis sampling in a freely-moving rat model (59). *In vivo* probe recoveries were determined using the retrodialysis of a rhodamine analog, and results show that cyclosporin A enhances the distribution of rhodamine-123 to the cortical ECF 3 to 4 fold, presumably by inhibiting the p-glycoprotein pump at the level of the blood-brain barrier (59). Burgio et al. also used microdialysis to study the effect of cyclosporin A on the distribution of etoposide to the cortical ECF, using retrodialysis of etoposide itself prior to intravenous administration of the drug to calibrate probe recovery (93). This study showed increased levels of etoposide in the cortical ECF upon cyclosporin A administration, however, the plasma levels of etoposide increased in proportion to the brain increase. This result may indicate that the increase in etoposide in the brain is due to a decrease in the systemic clearance of etoposide, and not necessarily an inhibition of the p-glycoprotein efflux pump at the blood-brain barrier (93).

Anticonvulsant Agents

Microdialysis sampling has been utilized in numerous studies that have examined the neuropharmacokinetics of anticonvulsant agents in both experimental animals and in humans (94–96). The microdialysate concentration of valproic acid has been determined in the rat brain ECF by Wolf *et al.*, however, they did not use *in vivo* calibration of the probe recovery (97). Golden *et al.* examined the effect of probenecid on the serum-CSF transport of valproic acid in rats using microdialysis (98). An increase in the Michaelis constant for efflux was observed with probenecid treatment and long term valproic acid infusion, which the authors state suggests competitive inhibition of transport by probenecid and valproic acid metabolites (98). However, this study also did not use *in vivo* recovery values to correct the dialysate concentrations, which may affect the quantitative accuracy of the measurements.

Welty et al. have used microdialysis to determine the brain ECF concentration-time profile of a novel GABA derivative, gabapentin in anesthetized rats (99). Probe recovery values

were determined *in vitro*, and the authors state that for the purposes of the study, to characterize the time-course of drug in brain to the anticonvulsant effect, the microdialysis method offers a suitable estimate of brain ECF concentrations. It was found that the anticonvulsant effect of gabapentin was delayed by time-dependent events other than the distribution of gabapentin to the brain (99). In another gabapentin microdialysis study from the same group, the bidirectional permeabilities of gabapentin across the blood-brain barrier were determined using a more quantitative experimental approach coupled with pharmacokinetic modeling of the transport processes (100). In this study, conscious restrained rats had *in vivo* probe recovery determinations done by retrodialysis of radiolabeled gabapentin prior to the intravenous infusion of the drug. Moreover, the time course of gabapentin in brain tissue homogenates was determined independently to validate model assumptions. The blood-brain barrier efflux and influx clearances of gabapentin were determined using a hybrid pharmacokinetic model that assumes that the transport between the intracellular and extracellular space in the brain is much more rapid than the transport across the blood-brain barrier. Using this model, it was found that the efflux clearance was substantially greater than the influx clearance and that the total brain concentration of gabapentin was significantly higher than the ECF concentration; a consequence of which would be an underestimated efflux clearance if using the traditional tissue-homogenate pharmacokinetic approach (100).

The blood-brain barrier transport of another GABA derivative, baclofen, has been studied using brain microdialysis sampling in anesthetized rats (101). The probe recoveries were determined using the antipyrine reference method described by Terasaki *et al.* (69). The results of this study are similar to the results found with gabapentin, i.e., baclofen efflux clearance through the blood-brain barrier is greater than the influx clearance (96). Moreover, studies with probenecid indicate the efflux transport of baclofen is inhibited by the coadministration of probenecid (101), a known inhibitor of the organic anion transport system.

The brain distribution of carbamazepine and its epoxide metabolite has been studied by microdialysis in freely-moving (102) and anesthetized rats (103). Van Belle et al. studied the distribution of both carbamazepine and its epoxide in different regions of the rat brain (103). In this study an analog of carbamazepine, m-carbamazepine, was used a reference calibrator to determine probe recovery *in vivo* by retrodialysis (104). Both carbamazepine and the metabolite were homogeneously distributed to the hippocampus and cerebellum, with the brain ECF-to-blood AUC ratio equal to unity for the parent compound and approximately 0.45 for the metabolite.

Kurata *et al.* have studied the distribution of trimethadione and its major metabolite in the blood, liver and brain using microdialysis in the freely-moving rat (105). No attempts were made in this study to correct for probe recovery in each of the different tissues, where different diffusivities of the parent and metabolite may remarkably influence the recovery ratio.

Other CNS Active Agents

Some of the initial neuropharmacokinetic studies to utilize microdialysis have studied the brain distribution of methylxanthine compounds, such as caffeine and theophylline (6,106–108). Stahle *et al.* have correlated the brain concentration of theophylline to activity levels in freely-moving rats (6). Stahle has also shown in relative recovery studies using caffeine and theophylline, that compounds that may be very similar structurally, e.g., caffeine and theophylline, may have significantly different recovery values depending on the tissue studied. The important consequence of this is that *in vivo* recovery validation needs to be done for each tissue type and each calibrator (106).

The distribution of aluminum to the brain and liver has been studied by Yokel et al. using microdialysis sampling in rats and rabbits (109,110). Allen et al. determined the brain/blood aluminum concentration ratios in anesthetized rats (111) using microdialysis sampling. They found the aluminum brain/blood concentration ratio to be approximately 0.1 to 0.15, over a wide concentration range. Moreover, upon addition of cyanide to the probe perfusate, the brain/blood concentration ratio increased to unity. These results suggest the presence of an efficient, energy-dependent carrier system for aluminum out of the brain (111).

The distribution across the blood-brain barrier of the anticholinesterase drug, tacrine, has been studied in awake, freely-moving rats using microdialysis sampling of the brain and blood (112,113). Telting-Diaz and Lunte measured tacrine dialysate concentrations from a probe placed in the hippocampus of the rat, using *in vitro* recovery determinations to calculate hippocampal ECF tacrine concentrations (112). The authors state that they found that tacrine rapidly penetrated into the brain, and that the hippocampal levels were ten times lower than the total plasma concentrations (112). However, Brundage and Sawchuk have shown that, using 3-methyltacrine as a retrodialysis calibrator *in vivo*, the levels of tacrine in the CSF and the cortical ECF were not significantly different than the free plasma levels (113). The observed differences in these two studies may be due to differences in recovery between *in vitro* and *in vivo* measurement, with the higher *in vitro* recoveries leading to an underestimation of the true tacrine ECF concentration. Because of the different methods to determine probe recovery, it is unclear that the difference in estimated tacrine brain ECF/plasma concentration ratio between these studies could be explained by the fact different sites within the brain were measured, even when correcting for free fraction in plasma. In another study where tacrine concentrations were not measured, microdialysis sampling was used to measure the local pharmacodynamic effects of tacrine delivered via the microdialysis probe on dopamine levels in the striatum of the freely-moving rat model (114) illustrating the use of the microdialysis probe to deliver drugs to local sites of action and directly measure pharmacologic effect.

Microdialysis sampling has been used to determine the simultaneous blood and brain pharmacokinetics of SDZ-ICM-567, a 5-HT3 antagonist, in freely moving rats (115). The *in vivo* probe recovery was determined using the zero-net-flux method, and the blood microdialysis data were validated by obtaining periodic blood samples and measuring the blood concentrations by HPLC and determining the SDZ-ICM-567 free fraction in blood by equilibrium dialysis (115). The pharmacokinetic parameters obtained by the blood microdialysis agree with those obtained from the whole blood experiments and the free concentration of SDZ-ICM-567 in the brain represented approximately 19% of the free concentration in blood, sug-

gesting an active transport out of the central nervous system (115).

Sato *et al.* have examined the feasibility of brain microdialysis for the study of imipriamine neuropharmacokinetics (116). Imipriamine and its major metabolite, desipramine, were measured in dialysates from the striatum of freely-moving rats (116), and concentrations were reported without correction of probe recovery. The coadministration of SKF-525A, an enzyme inhibitor, significantly prolonged the half-life of imipramine in the brain and decreased the fraction of imipramine converted to desipramine (116).

Malhotra *et al.* have examined the distribution of an experimental NMDA antagonist, EAB 515, to the cortical ECF and the CSF in freely-moving rats using microdialysis sampling (47,117,118). The *in vivo* recovery of the microdialysis probes was determined by simultaneous retrodialysis using a hydroxylated analog of EAB 515 as the calibrator. The use of this calibrator for retrodialysis recovery determination was validated in experiments using the zero-net-flux technique. EAB 515 was administered by intravenous infusion and by intracerebroventricular infusion in a crossover fashion in some rats. After the constant rate infusion of EAB 515, the concentration ratio of EAB 515 in CSF to plasma was approximately 18%, whereas the ratio of cortical ECF to plasma EAB 515 concentration was approximately 8% (47). Upon ICV administration of EAB 515 to the lateral ventricle, a significant distribution advantage to the cortical ECF over intravenous administration (over 100 fold) was observed. These results indicate a significant contribution from the CSF-brain transport pathway to the distribution of EAB 515 to the cortical ECF (47). Because these studies were conducted over a relatively long period (up to approximately 1400 minutes), the possibility of changes in probe recovery over time cannot be discounted. This was evaluated by investigating trends in recovery during the experimental period. Figure 6 depicts results in a typical rat employing retrodialysis of a structurally similar analog of EAB 515 over the experimental period. No trend in recovery (loss of calibrator) was noted.

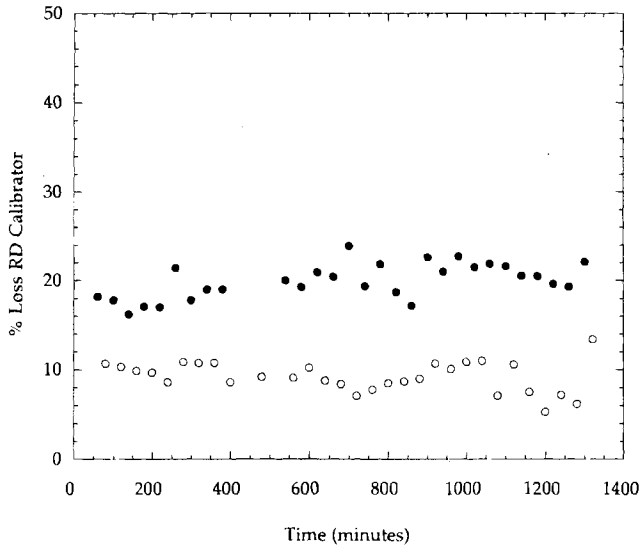

Fig. 6. The time-course of *in vivo* probe recovery of the NMDA antagonist, EAB 515, as determined by retrodialysis, in cortical ECF (solid symbols, 2-mm probe) and ventricular CSF (open symbols, 1-mm probe) in a typical rat.

Miscellaneous CNS Agents

The use of intracerebral microdialysis to determine changes in blood-brain barrier transport characteristics following hypertonic mannitol infusion has been examined by de Lange et al. (119). Atenolol was used as the model drug and the unilateral opening of the blood-brain barrier was achieved by infusion of a hyperosmotic mannitol solution (25% w/v) into the left carotid artery of rats. Changes in blood-brain barrier permeability to atenolol was expressed as the ratio of the AUC in the brain extracellular fluid to the AUC in plasma (119). This ratio was three times higher for the mannitol treated hemisphere as compared to the contralateral hemisphere or following infusion of saline as control. The authors state that these results are further evidence that intracerebral microdialysis is capable of revealing changes in blood-brain barrier permeability and that regional and time-dependent changes in drug concentrations in the brain can be demonstrated using this technique (119). In another recent study from the same group, the regional distribution kinetics of drugs in the rat brain was examined using the microdialysis technique (120). Atenolol and acetaminophen were used as model compounds and two transverse microdialysis probes were placed in parallel in the frontal cortex of the rat, one probe employed as a drug delivery device and the other as a drug sampling probe. Following *in vivo* recovery determination, brain ECF drug concentrations were found to be dependent on the drug and the interprobe distance. Moreover, a model was developed to estimate the ratio (transfer coefficient from brain to blood/effective diffusion coefficient of drug in ECF). This ratio was greater for acetaminophen, the more lipophilic drug (120).

PERIPHERAL TISSUES AND ORGANS

Subcutaneous Adipose Tissue

Many reports have described the application of microdialysis in preclinical investigations of lipolysis in subcutaneous adipose tissue. Some of these studies have been performed to investigate the utility of microdialysis in monitoring subcutaneous glucose in animals, and in man. Arner and Bolinder have reviewed the microdialysis of small endogenous molecules in adipose tissues (121).

Jansson *et al.* compared the zero-net flux method for probe recovery calibration *in vivo* with a simple isotopic method using rat epididymus fat pads (122). The recovery calculated by both methods were not significantly different. The authors concluded that the methods yielded comparable results, and the more convenient isotopic method was preferred for determining adipose tissue interstitial recovery for glucose, lactate and glycerol.

Capillary ultrafiltration (123) differs from microdialysis in that it produces an ultrafiltrate of tissue extracellular fluid rather than a dialysate. Recoveries are thus assumed to be 100% with reference to the unbound or "free" analyte concentration in the extracellular fluid. Capillary ultrafiltration probes were implanted in subcutaneous tissue in awake rats, and employed to monitor the disposition of acetaminophen and theophylline. Ultrafiltration probes provided samples at a rate of 2–3 μL/min which were analyzed by liquid chromatography. Simultaneous ultrafiltration and microdialysis probes and multiple ultrafiltration probes were used in individual animals for method valida-

tion. The capillary ultrafiltration probes yielded a constant flow rate of 2.4 μL/min, withdrawing 50 nL/min/mm of fluid from the extracellular space. This method was shown to be an excellent tool for monitoring drug disposition in conscious animals without the need for blood sampling. A major theoretical advantage of this technique is that it does not require calibration of relative recovery.

The effects of acute and chronic ethanol dosing on acetaminophen pharmacokinetics were studied in freely-moving rats (124). Loop-type microdialysis probes with membrane lengths of 40–60 mm, which afforded high recoveries and good precision at perfusate flow rates less than 2 μL/min, were used. Microdialysis sampling of drug levels in subcutaneous tissue and blood by microdialysis provided excellent results for acetaminophen. Acute intraperitoneal doses of ethanol increased the relative bioavailability, measured as AUC, by 40%. Changes in elimination half-life, CL and Vd were also observed. Larger doses of ethanol, up to 2 mL/kg, had a similar effect in some animals; reduced absorption and altered elimination parameters in others were attributed to apparent decreases in abdominal blood flow. The effects of chronic doses of ethanol (5% in drinking water for 2 weeks) were also examined using microdialysis.

Skin

A microdialysis study of *in vivo* transdermal absorption of methotrexate (MTX) in rats with or without a new penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101) has been reported (125). A solution composed of 2.5 mM MTX and 3% (w/v) HPE-101 was applied to the shaved abdomen, in which a semipermeable membrane cannula of 10-mm length was inserted intracutaneously. Intradermal microdialysis was performed at a flow rate of 1 μL/min for 12 hr. The concentration of MTX in the dialysate was measured by fluorescence polarization immunoassay (FPIA). 3% HPE-101 increased the mean dermal MTX concentration from 0.06 μM (control) to 56 μM in the dialysate from 8 to 12 hr. HPE-101 at lower concentrations also increased the total recovery of MTX in dermal dialysate.

Microdialysis sampling for valproic acid (VPA), was evaluated *in vitro* in an experimental first-order elimination system (126). The elimination rate constant of VPA in dialysate was in good agreement with that determined by direct sampling. The effect of HPE-101 on the transdermal absorption of VPA was examined in rats *in vivo* using microdialysis. Intradermal microdialysis was performed for 7h following dermal application of 50 mM VPA solution with or without 3% HPE-101. The authors concluded that the transdermal absorption rate of VPA was increased 80-fold by the enhancer. Microdialysis was found to be reliable in assessing the *in vivo* transdermal absorption of VPA.

The relative microdialysis recovery of 5-fluorouracil (5-FU) was found to be affected by pH but not by donor concentration (127). To confirm this a study comparing direct and microdialysis sampling of a diffusion cell receptor compartment was performed. The 5-FU concentration and pH changed substantially, but recovery of 5-FU was not adversely affected. Flux from a topical preparation of 5-fluorouracil was monitored utilizing microdialysis in excised rat skin *in vitro*.

Ault *et al.* employed microdialysis sampling of the dermis *in vivo* using a linear microdialysis probe which had no effect on the flux of drug through the skin *in vitro* (128). The extent of tissue damage *in vivo* due to probe implantation was evaluated by histological examination and microdialysis delivery studies. Tissue damage due to implantation was minimal, and infiltration of lymphocytes into the tissue, observed beginning 6 hours after probe implantation, had no effect on the probe function. Scar tissue appeared at approximately 32 hours. Delivery of 5-fluorouracil (5-FU) ranged from 20 to 25% for different probes implanted in different animals (n = 6) and showed good reproducibility. Constant-rate transdermal delivery was measured for at least 24 hours. The dermal concentration of 5-FU from a topically applied cream was continuously monitored, and was approximately 40-fold higher for excised skin *in vitro* than for intact skin *in vivo*.

Because microdialysis probes with large surface areas (20–100 mm membrane length) provide increased relative recovery over conventional shorter probes (1–4 mm), long "loop-type" probes may offer enhanced relative recovery when implanted subcutaneously. Effects on relative recovery of membrane area, probe size, inlet and outlet tubing dimensions, collection rate, bulk flow through the membrane and flow-rate were studied (129). Polyacrylonitrile and regenerated cellulose membrane fibers with different geometries were examined. Sampling fibers 30–100 mm long were used. Probes with large membrane surface areas provided relative recovery of greater than 50% at flow-rates greater than 5 μL/min.

Skeletal Muscle

As in the case of adipose tissue, a variety of studies have employed microdialysis to examine interstitial fluid concentrations of glucose, lactate, amino acids, and other endogenous compounds in skeletal muscle. Some of these have used microdialysis to investigate the effects of pharmacological agents on the levels of these compounds in muscle extracellular fluid. Recently, reports which deal with the distribution of drugs and metabolites to skeletal muscle in animals have begun to appear.

Using a capillary "tube" model for clearance, steady-state dialysis kinetics were related to microdialysis recovery from muscle extracellular fluid, as well as from buffer and erythrocyte suspension (10). The dialysis clearance was related to dialysis flow rate, F, and the permeability rate constant, PA, for microdialysis performed with a linear microdialysis probe. An effective dialysis coefficient (Rd), was defined as the ratio of the *in vivo* PA and *in vitro* PA, and used to explain differences between *in vivo* and *in vitro* microdialysis recoveries. Antipyrine permeability coefficients were characterized by an activation energy of 5.5 kcal/mol. over the range of 15–37 C°. Good correlation was observed between the reciprocal of the permeability rate constant and the square root of MW in the range of 18–1039 D. MW was not a determinant of Rd in the erythrocyte suspension. The *in vivo* permeability coefficients were measured in muscle for [^{3}H]water, [^{14}C]urea, antipyrine and [^{14}C]sucrose at steady state. No significant difference of Rd in muscle tissue was demonstrated for these four model substances. Based on their results, the authors propose an equation to relate the concentration in the dialysate and the interstitial fluid at steady-state.

Another study examined the pharmacokinetics of intravenously administered levodopa (L-dopa) in plasma and skeletal muscle (130). A single intravenous dose of L-dopa (25 mg/kg) was given to an anesthetized beagle dog. L-dopa and its O-methyl metabolite, 3-O-methyldopa (3-OMD), were monitored in plasma and skeletal muscle simultaneously by microdialysis for 3 hr following dosing. Mean Cmax values for L-dopa in plasma and skeletal muscle extracellular fluid were 173 and 14.6 ng/mL, respectively. The areas under the plasma concentration-time curve (AUCs) for L-dopa were 20 times higher in plasma than in muscle. No distribution equilibrium for L-dopa was reached between the two tissues during the study period.

In a related study, the effects of carbidopa (CD) and entacapone pretreatment on the pharmacokinetics and metabolism of L-dopa were investigated, using microdialysis of plasma and skeletal muscle extracellular fluid, in the beagle dog (131). CD had no effect on plasma L-dopa levels, which declined biexponentially following intravenous dosing with L-dopa. In contrast, a monoexponential decline in L-dopa levels in muscle ECF was seen. CD pretreatment caused an increase in the area under the plasma concentration-time curve (AUC) and elimination half-life of L-dopa in muscle extracellular fluid and increased the accumulation of 3-O-methyldopa (3-OMD) and dopamine (DA) in muscle extracellular fluid. CD decreased the levels of L-3,4-dihydroxyphenylacetic acid in both plasma and in muscle extracellular fluid. Entacapone inhibited the formation of 3-OMD, resulting in a reduction of its AUC in plasma and muscle extracellular fluid.

Eye

Distribution of drugs from blood to the vitreous is generally poor. However, since penetration into this space is considered to be important for many therapeutic agents, microdialysis has been used to measure the efficiency of drug delivery to the eye.

Ben-Nun *et al.* described the fabrication, surgical implantation, and use of hollow-fiber probes in studying vitreal concentrations of gentamicin following subconjunctival or vitreal injection of the antibiotic in cats (132). The investigators used *in vitro* calibration to assess recovery, and by direct visualization of the point of intravitreal injection through the dilated pupil, they were able to relate vitreal concentrations to the injection site.

Intravitreal microdialysis sampling of gentamicin concentrations following intravitreal bolus administration of the aminoglycoside in cats with bacterial eye infections has been reported (133). Control eyes and those with bacterial-induced endophthalmitis were studied. A one-compartment model which considered diffusion to the sampling site and elimination from the vitreous was adopted. Elimination rate constants were greater in infected eyes than in controls 0.11 vs. 0.055 hr^{-1}, and this difference was attributed to increased gentamicin permeability in the blood-retinal barrier in endophthalmitis.

Waga *et al.* reported the use of an intraocular dialysis probe made from a soft tube with the dialysis membrane mounted in a fenestrated protecting sleeve (134). It is claimed that these probes are functional in the eye for several weeks or more, and thus permits the investigator to avoid short-term studies where acute surgical trauma may affect drug concentrations in the vitreous.

Stempels *et al.* have described a microdialysis system with a removable probe and a fixed scleral entry port for use in the rabbit eye (135). The authors state that the probe can be inserted several times with little trauma, allowing repeated sampling in the same animal. This system was utilized for the measurement of dopamine, dihydroxyphenyl acetic acid, and noradrenaline in the vitreous of healthy rabbits. It is suggested that it may also be used for studies of the distribution of therapeutic agents into the eye. These investigators (136) have used intravitreous microdialysis to examine the effect of retinal laser coagulation on catecholamine levels.

Waga and Ehinger (137) implanted microdialysis probes intraocularly in rabbits and perfused them with three groups of agents: antibiotics (benzylpenicillin and cefuroxim); corticosteroids (triamcinolone and dexamethasone); and cytostatics (daunomycin and 5-fluorouracil). They also examined three substances of differing molecular weights: formic acid (MW 70), glucose (MW 189) and inulin (MW ca. 5200). All substances except inulin diffused readily through the polyamide membrane. They observed that polyamide membranes interacted less with lipophilic agents than did polycarbonate membranes. The latter material was therefore used in their intraocular perfusion studies to deliver therapeutic agents to the vitreous body of rabbits through implanted microdialysis membranes.

Hughes *et al.* (138) developed a novel microdialysis-perfusion technique to examine the elimination of the antiviral nucleosides, ganciclovir and acyclovir, from the vitreous humor of the rabbit eye. Vitreous elimination of the antivirals was rapid, and differed between pigmented and albino strains. An important methodological finding in this study was that probe relative recoveries of the nucleosides in a buffered saline solution were no different from those determined in vitreous humor *in vitro*, in spite of the greater viscosity of the vitreous. The authors explain this result by saying that the microviscosity is more important to solute diffusion in the vitreous than macroviscosity, and in the absence of any osmotic, pressure, or thermal gradients, the relative recovery of the probe in the vitreous and buffer solutions will depend only on the temperature of the medium (138). Therefore, *in vitro* recoveries were used to calculate the nucleoside concentrations in the vitreous.

Heart/Lung

Kuzmin *et al.* (139) employed microdialysis in dogs to measure cardiac interstitial fluid concentrations of allopurinol and its metabolites, as well as the adenine nucleotide metabolites inosine, hypoxanthine, xanthine, and uric acid. Animals received 1 and 10 mg/kg allopurinol intravenously. The short half-life (1.8 min) for drug penetration into the heart was dose-independent; however the cardiac interstitial fluid clearance was much reduced for the higher dose, perhaps due to capacity-limited conversion of allopurinol to oxypurinol by xanthine dehydrogenase/oxidase.

In a study by Lonnroth *et al.* (140), microdialysis was performed on the left ventricular wall in six pigs which had received a priming intravenous injection of 5 mg of propranolol supplemented by a constant-rate propranolol infusion for 40 min (5 mg propranolol per h). Maximum concentrations of propranolol (mean $\pm$ SD) were 97 $\pm$ 29 and 6 $\pm$ 2 nM in plasma and in interstitial water, respectively. *In vivo* calibration was used to determine the extracellular fluid levels of the drug.

Eisenberg and colleagues (141) utilized microdialysis to investigate the concentration versus time profiles of tobramycin and gentamicin in the lung epithelial lining fluid in the anesthetized rat. After the aminoglycosides were administered intravenously as a bolus, mean (n = 5) penetration ratios into the ELF were determined and found to be 0.36 for gentamicin and 0.56 for tobramycin. The authors suggest that this technique can be useful for the intrabronchial measurement of drugs in the lung.

Blood/Bile

Scott and Lunte (142) reported the use of a flow-through microdialysis probe for sampling bile while maintaining normal bile flow in anesthetized rats. Three sites (bile, blood, and liver extracellular fluid) were simultaneously and continuously sampled in a single experimental animal. After intravenous infusion of phenol, the major hepatic metabolite was found to be phenyl-glucuronide. Hydroquinone and 2-glutathionyl-hydroquinone were also detected. The authors concluded that the metabolites are actively secreted into the bile since their biliary concentrations were higher than liver concentrations (142).

Ekstrom *et al.* (143) used microdialysis to investigate tissue exposure to methotrexate (MTX). Probes were implanted in the jugular vein, femoral muscle, and liver of anesthetized male Wistar rats. MTX was given as an intravenous bolus injection (100 mg/kg) and blood samples were obtained for a total of 6 h. Total and unbound plasma concentrations and microdialysis effluent from tissue and venous probes were analyzed by HPLC. Binding of MTX to plasma proteins was 21% *ex vivo* and 23% *in vivo*. *In vitro* microdialysis of this spiked plasma resulted in 23% relative recovery of the unbound fraction, whereas the relative recovery from venous microdialysis probes was 18%. Ultrafiltrate MTX concentrations in the blood correlated well with venous microdialysis perfusate concentrations, as did total MTX concentrations in venous blood with the drug levels in microdialysis samples from muscle and liver. Area under the curve estimations yielded an MTX exposure of 30% and 46% for the muscle and liver relative to blood (143).

Intravenous microdialysis has been used to examine glucose concentrations in freely moving rats (144). Microdialysis recovery of glucose was measured *in vitro* (22.6 ± 1.0%) and heparinized animals *in vivo* (18.1 ± 1.2%) and was found to be similar. However, in non-heparinized animals the *in vivo* recovery was significantly reduced (9.5 ± 2.1%) (144). A unique feature of this study was that the intravenous probe was placed in the right atrium via the jugular vein.

Evrard *et al.* (145) describe a new microdialysis probe design for intravenous sampling in the vena cava of the rat and the mouse. They examined the pharmacokinetics of flurbiprofen using *in vivo* retrodialysis of flurbiprofen before the experiment to determine probe recovery (145). Their elegant studies indicate that microdialysis sampling in the blood can be used for pharmacokinetic studies in both the rat and the mouse, which could have important implications in reducing the number of small laboratory animals required to complete pharmacokinetic and toxicokinetic analyses, while vastly improving the quality of the data collected.

Liver

Van Belle *et al.* (103) examined blood and liver distribution kinetics, a metabolic interaction, and hepatic metabolism of carbamazepine (CBZ) in the rat, using microdialysis with *in vivo* calibration. The disposition of CBZ and its epoxide metabolite (CBZE) in blood and liver, after a single dose of CBZ, was studied in control animals and in rats after pretreatment with clomipramine. Measurements of blood AUCs of both parent and metabolite by microdialysis demonstrated inhibition of the epoxidation pathway by clomipramine. The authors also administered CBZ into hepatic extracellular fluid via the microdialysis probe and found that the formed CBZE concentration was approximately 19% of the administered CBZ concentration (103).

Kurata *et al.* (146) used microdialysis to quantitate antipyrine in liver and blood of rats. Probes were implanted in the liver and right jugular vein and antipyrine was administered intraperitoneally (50 mg/kg). Concentrations of antipyrine in dialysate from the two probes were compared with those in blood drawn from the left jugular vein. The elimination half lives in serum, blood dialysate and hepatic dialysate were not significantly different (2.2, 2.1, and 2.1 hrs, respectively). Although the concentrations of $Na+$, $K+$ and $Cl-$ in the liver dialysate were constant for no more than 12 hrs in anesthetized rats, concentrations of these ions in the dialysate were stable for at least 48 hrs in freely moving, conscious animals. The authors concluded that microdialysis should yield useful information related to drug levels in rat hepatic extracellular fluid for up to 48 hrs (146).

Yokel and colleagues (110) studied the effect of intravenous desferrioxamine (DFO) on the mobilization of aluminum (Al) into blood, brain and liver extracellular space in the Al-loaded rat by microdialysis. When corrected for probe recovery, liver extracellular fluid Al peaked at approximately 1400 microgram/l whereas maximum blood and brain extracellular fluid Al were 860 and 170 microgram/l, respectively. The observed rapid rise in hepatic extracellular Al above blood levels following DFO suggests that DFO distributes rapidly from the vascular compartment to form an Al-DFO complex which extracts Al from hepatocytes. The authors did not observe a decline in brain extracellular Al following DFO dosing, and suggested this may be due to its inability to diffuse into blood against a concentration gradient (110).

Scott *et al.* (147) investigated the pharmacokinetics of acetaminophen in the blood and liver of an anesthetized rat. The concentration-time profiles of the sulfate and glucuronide metabolites as well as acetaminophen were monitored using microdialysis sampling. In their microdialysis studies of the hepatic metabolism of phenol, Scott and coworkers (148) were among the first to point out that this technique could yield more complete metabolic and pharmacokinetic data by intensive sampling in a small number of animals. The results of their studies in rat liver in situ, when compared with experiments using hepatic microsomes *in vitro*, showed major differences (148).

Protein Binding

Microdialysis sampling has been used for the *in vitro* determination of plasma protein binding of drugs (17,149–151), and experimental results have compared favorably with standard equilibrium dialysis (149) and ultrafiltration techniques (17). The microdialysis method may have some advantages for *in vitro* plasma protein binding studies in that the volume of

the sample is constant and the concentrations of the components of the sample do not change greatly during the experiment (17). Moreover, the temperature of the sample can be more precisely controlled using microdialysis versus separation techniques involving centrifugation, such as ultrafiltration and ultracentrifugation. Therefore, the microdialysis method may provide a means of determining the thermodynamics of the binding equilibrium (18). Problems of nonspecific adsorption to membranes and devices used to separate the bound from the free drug are less severe with the microdialysis method because of the much reduced surface area of the membrane. The plasma binding of drugs that have a high degree of nonspecific binding, cyclosporin A (152) and amphotericin B (153), have been determined using microdialysis techniques. However, as with any microdialysis experiment, the accurate determination of the probe relative recovery in the sample matrix is critical for valid protein binding determinations. Nakashima *et al.* (154) have shown decreasing *in vitro* relative recoveries for probes placed in physiological saline, albumin solutions and plasma, respectively.

In vitro drug binding studies can be corroborated with drug binding *in vivo* using microdialysis determinations in the blood and simultaneous blood sampling from unanesthetized animals (115,151,155). Correlation can be made between the *in vitro* and *in vivo* binding of drugs in the blood (115) or plasma (154–157), depending on which total drug concentration (plasma or blood) is measured following blood sampling. Using microdialysis to determine blood binding will account for drug taken up by blood cells, which can be an important determinant of free drug concentration (158). Dubey *et al.* (156) report that the *in vitro* binding of diazepam in blood, as measured by microdialysis, was similar to that measured by microdialysis *in vivo* in the vena cava. Their study confirms, perhaps for the first time, the validity of extrapolating countless *in vitro* and *ex vivo* binding studies to the *in vivo* situation. Evrard *et al.* (155) have compared the *in vitro*, *ex vivo*, and *in vivo* binding of flurbiprofen in the rat. They have shown, using simultaneous blood sampling and *in vivo* microdialysis sampling, that the *ex vivo* plasma protein binding studies are consistent with the *in vivo* results from the microdialysis experiments. However, the *in vitro* results systematically underestimated the unbound fraction *in vivo*. One explanation given for this discrepancy is the plasma spiked with the drug for the *in vitro* studies may have different characteristics than the experimental animal's plasma that may affect binding, such as different protein concentrations. Moreover, the *in vitro* technique neglects the potential competition between metabolites and the parent compound for binding sites. Determining the free fraction of a drug *in vivo* overcomes many of these possible confounding variables and microdialysis sampling is useful in accurately determining the free concentration vs. time profile.

Pharmacokinetic Studies in Man Using Microdialysis

Numerous reports have described the application of microdialysis in man. The majority of these have examined subcutaneous extracellular fluid concentration of endogenous agents, however, recent publications report the clinical use of microdialysis investigating tissue kinetics of antibiotics (159,160), antitumor agents (161), and nicotine (162). Some of these studies have investigated the kinetics of change of these agents when they are given exogenously (163). For example, glucose, lactate, pyruvate or glycerol kinetics in subcutaneous adipose tissue have been studied after oral (164,165) or intravenous (166) glucose administration. The use of microdialysis in evaluating adipose tissue metabolism in humans has been reviewed (167). Recently, studies using microdialysis in man to examine the central nervous system distribution of drugs have appeared. These have been conducted primarily in epileptic or stroke patients undergoing brain surgery.

Subcutaneous/Adipose Tissue

Lonnroth *et al.* (140) examined the unbound concentration of propranolol in the interstitial space (periumbilical subcutaneous tissue) *in vivo*, in seven males by microdialysis, employing *in vivo* calibration. Ten hours after a dose of 80 mg of propranolol, mean total plasma and free interstitial propranolol concentrations were 80 and 7 nM, respectively. After a second dose, maximum concentrations in plasma and interstitial water was reached at approximately 1.5 hr, and the mean concentrations in these sites were 594 and 27 nM, respectively. These results indicate that microdialysis is a useful tool for monitoring unbound concentrations of a drug in the extracellular space (140).

In an investigation of extracellular insulin and inulin concentrations, microdialysis samples from the abdominal subcutaneous tissue were obtained at 20-min intervals during constant-rate administration of these agents (168). Microdialysis recovery was determined in situ at steady state. Mean extracellular and plasma insulin concentrations were estimated as 654 and 1176 pM, respectively, i.e., a 44% difference ($P < 0.001$). A doubling of the insulin infusion rate increased the extracellular insulin concentrations more slowly than those in plasma. At the new steady state the relative concentrations in both sites were similar. However, extracellular inulin levels were similar to those in plasma in subjects receiving a constant-rate inulin infusion. The authors conclude that tissue clearance of insulin, in conjunction with an endothelial barrier for insulin in the subcutaneous tissue results in lower insulin levels in the extracellular fluid than in plasma (168).

Stahle *et al.* (169) used microdialysis to estimate extracellular levels of caffeine in subcutaneous abdominal adipose tissue in volunteers following oral administration of 5 mg/kg of caffeine. The peak extracellular levels were in the range of 20–80 microM. Although the time-course in blood and extracellular fluid were similar, plateau concentrations were not closely correlated. The estimated mean concentration of five individuals was similar in blood and extracellular fluid. Results showed that microdialysis provided useful data on drug distribution in man, and that adipose tissue levels of caffeine did not closely mirror concentrations in blood (169). Caffeine levels in the extracellular fluid were too small to affect phosphodiesterase but were sufficient to inhibit at the adenosine receptor.

In an attempt to examine the limitations of microdialysis in pharmacokinetic studies using human tissues, Muller *et al.* (170) inserted microdialysis probes into the medial vastus muscle or the periumbilical subcutaneous adipose layer of 13 healthy volunteers. These subjects then received either acetaminophen (1 g orally) or gentamicin (160 mg, as an intravenous bolus). Concentrations of these agents were monitored in plasma, muscle, and subcutaneous tissue (170). Calibration of

the microdialysis probes was carried out *in vitro* and *in vivo* by retrodialysis. The authors were able to obtain continuous concentration versus time profiles in muscle and subcutaneous tissue. Primary determinants of the pharmacokinetics (absorption half-life, elimination half-life, maximum concentration, time to maximum concentration, area under the curve) were determined for muscle and subcutaneous extracellular fluids; tissue extracellular fluid/plasma concentration ratios were also determined. The same group report a similar study investigating theophylline kinetics in humans (171). In both studies, the reproducibility of tissue drug concentration measurements was good, demonstrating that microdialysis sampling is reliable in the measurement of drug concentrations in human muscle and subcutaneous tissues.

Bradykinin release in patients undergoing surgical removal of impacted molars has been examined using microdialysis (172), with probes implanted in the surgical area immediately following surgery. Interestingly, the investigators were able to observe an attenuation in bradykinin levels when patients were pretreated with methylprednisolone, and found a hysteresis in the pain score-bradykinin relationship in both glucocorticoid and placebo-treated patients.

In an elegant study of urea kinetics during and following hemodialysis, Metry *et al.* (173) used subcutaneous tissue microdialysis to characterize the postdialytic rebound in urea levels. These investigators were able to relate the extent of this rebound to the fractional reduction of the urea levels in plasma during hemodialysis. Probe calibration *in vivo* was achieved by the method of zero-net flux.

Microdialysis may also be performed in human skin to assess cutaneous drug levels during transdermal delivery. To evaluate cutaneous nicotine levels, Hegemann *et al.* (174) employed a transdermal delivery system containing 35 mg of nicotine. Nicotine levels were measured using HPLC in the dialysate of human skin. Studies *in vitro* demonstrated that nicotine levels in the dialysate correlated with those in the dialyzed medium. In nine male volunteers receiving nicotine by transdermal delivery, nicotine was detectable within 90–180 min, and peak levels of approximately 1 μg/mL were detected within 240–360 min of transdermal patch application. Although nicotine kinetics were found to be independent of skin barrier function, the detectable maximum nicotine levels appeared to depend on the location of the probe (174). This suggests that although transdermal microdialysis studies performed in crossover in the same subject may yield useful results, intersubject variability in probe placement could lead to variability in data which might limit the applicability of studies performed in a panel of subjects.

Anderson *et al.* (175) employed microdialysis to study the percutaneous absorption of ethanol. Probes were inserted via a guide into the skin of the ventral forearm in 7 volunteers. Ethanol (99.5%) was applied to the skin and the probe was perfused at a flow of 1 μL/min. Dialysate samples (50 μL) were analyzed by gas chromatography. Absorption of ethanol was observed in all subjects, with concentrations from the 9 probes inserted ranging from 10 to 800 μg/ml. Although calibration of probe recovery *in vivo* was not performed, the variation was attributed to inter-test or intersubject variability in ethanol absorption (175). The authors suggest that differences in metabolic capacity may also be important.

To determine if insertion of a microdialysis probe into the skin can affect reactivity of the skin test site or the measurement of substances in the skin, laser Doppler flowmetry was utilized to estimate cutaneous blood flow (176). In addition, laser Doppler perfusion imaging (LDPI), was used to study the time course of circulatory changes in the area of microdialysis probe insertion. LDPI was performed before, during, and after probe insertion in the skin of the ventral forearm in three subjects. Probe insertion produced an increase in skin blood perfusion in the entire test area. Within 15 min of probe placement, the circulatory changes were observed to center at the site of insertion and probe tip. Skin perfusion levels returned to normal levels within 60 min. Local anesthesia at the point of guide insertion did not affect circulatory reactivity in the adjacent skin (176).

De Boer and colleagues (177) employed a microdialysis probe for continuous transcutaneous sampling of ethanol and glucose across forearm skin stripped with cellophane tape. Concentrations of both compounds in the dialysate were measured on-line with continuous-flow analysis and compared with plasma values in human volunteers after ethanol consumption ($n = 4$) and oral glucose testing ($n = 5$), respectively. The dialysate and blood concentrations of ethanol and glucose were correlated in each subject although the dialysate-to-blood ratio varied among subjects. The recovery *in vivo* (mean) was 22.4 and 4.7% of the recovery *in vitro* for ethanol and glucose, respectively. Dialysate glucose concentration was found to be independent of blood flow. When the probe temperature was increased from 32 to 42°C, an increase in dialysate-to-blood glucose ratio was observed, consistent with the temperature-related increase in recovery *in vitro* (177).

Stahle and his colleagues (96) demonstrated the utility of microdialysis sampling of abdominal subcutaneous adipose extracellular fluid to monitor levels of valproic acid in 8 outpatients with epilepsy who were receiving valproic acid in daily doses of 600 to 3000 mg. Implanted probes were perfused at 0.3 to 0.4 μL/min. All patients were sampled hourly for 6 or 8 hr, except for one patient who was sampled for 3 days without problems. Blood samples were taken for the determination of total and free plasma valproic acid concentrations. Probes were not calibrated for recovery *in vivo* since the aim of the study was to explore the utility of microdialysis in routine clinical work in this patient group. Correlation between dialysate concentrations and free and total plasma concentrations of valproic acid was good in all patients. The investigators concluded that therapeutic drug monitoring employing microdialysis caused few side effects, since no inflammation or infection was noted, little pain was experienced during probe implantation, and the probes were not a hindrance to the patients (96).

Intravenous Microdialysis

Intravenous microdialysis has been proposed for monitoring patients requiring intensive care. Stjernstrom *et al.* (178) have used this technique for 24-hr periods of monitoring of levels of lactate, hypoxanthine, inosine, adenosine, glucose, creatinine and urea in patients, utilizing femoral, jugular, or medial cubital vein access. 20-mm microdialysis probes with an outer diameter of 0.9 mm were inserted via an intravenous catheter, and perfused at 2 μL/min. Recoveries *in vivo* of these small molecules were reported to be close to 100%, and it is suggested that these high values are due to the relatively large

surface area of the probe membranes and the high rates of mass transport in blood compared to that in extracellular fluids. The authors point out that continuous microdialysis in intensive care patients allows the characterization of fluctuations in levels of certain analytes such as glucose and lactate which might be missed using traditional sampling rates. It should be noted that these investigators used 60-min sampling periods, similar to those employed by Stahle *et al.* (96) to monitor subcutaneous extracellular fluid levels of valproic acid.

Brain

A number of investigations have employed microdialysis to examine concentrations of endogenous compounds in human brain. Kanthan *et al.* (179) have examined temporal lobe extracellular fluid levels of glutamate and other amino acids in a model of acute ischemia during the excision of tissue in patients treated for intractable epilepsy. During (180) has reviewed the neurochemical findings of studies involving microdialysis of the hippocampal region in epileptic patients. In addition to the studies which have focused on endogenous compounds in the central nervous system, a number of reports have described the application of microdialysis in investigating the distribution of drugs to the brain.

Scheyer *et al.* (95) studied the concentration of phenytoin concentration in human brain extracellular fluid using microdialysis. The study was performed in patients who were implanted with intracranial electrodes to investigate intractable epilepsy, with there phenytoin levels maintained at steady state. The probes had membranes which were constructed of regenerated cellulose (MW cutoff of 5000 D); these were 30 mm in length and 0.3 mm in diameter. The probes were advanced through the hippocampus, and were positioned with their tips in the amygdala. The investigators used changes in perfusate flow rate over a 10-fold range to calibrate recovery, using nonlinear regression analysis (2-parameter fit). The permeability-area product of these probes was found to be 1.2 μL/min, and recovery ranged from 30% to 80% when flow rates ranged from 2.5 to 0.25 μL/min. Concentrations of phenytoin in extracellular fluid were similar to unbound steady-state concentrations in plasma. No differences were noted between hippocampus and frontal sites (95).

Carbamazepine and carbamazepine-10,11-epoxide concentrations were determined by Scheyer and colleagues (94) using intracerebral microdialysis in three patients suffering from medically intractable epilepsy. Very small microdialysis catheters constructed of regenerated cellulose (30 mm in length and 0.3 mm in diameter) were fixed to and inserted with depth electrodes. These were use to sample drug and metabolite concentrations in anterior hippocampus extracellular fluid. Probe recovery was calibrated by varying the perfusate flow rates fro 0.25 to 2.5 μl/min, and estimating the permeability-surface area product (PA) according to the method of Jacobson (31). The authors found that the extracellular fluid concentrations of both carbamazepine and its metabolite were similar to their unbound concentrations in plasma. The method of calibration used in this study (extrapolation to zero flow rate) assumes that the effective PA, determined as a parameter in the regression analysis, is constant at all flow rates used in the study. Indeed, a decrease in this parameter might be expected at low flow rates. Parsons *et al.* (181) discuss the effect of PA on the estimated concentration at zero flow rate, and the consequences of PA being lower than that estimated by the model.

SUMMARY

Many applications of microdialysis have recently been seen in the field of pharmacokinetics, in particular in the study of drug distribution to the central nervous system. Recent advances which have come from disciplines such as the engineering sciences, polymer technology, theoretical pharmacokinetics, and bioanalytical chemistry have catalyzed the applicability of microdialysis into new areas in biology. There are, however, many vexing issues which remain to be addressed, some theoretical, and some practical.

Perhaps the most enigmatic difficulty in the application of microdialysis in pharmacokinetics is still the uncertainty in the relationship between dialysate concentration and that at the probed site, i.e., the controversy surrounding the assessment of relative recovery *in vivo*. Although many successful attempts to determine recovery have been realized using the zero-net-flux technique, this method is somewhat limiting as it requires the studied system to be at steady state. Several investigators, mostly in pharmacokinetic studies, have focused on the use of reference calibrators, such as that described here under the method of retrodialysis. In addition to interstudy variability of recovery there is a related question regarding time-dependency of recovery *in vivo* within an experiment. Both of these issues may be addressed, at least in part, by the continuous monitoring of probe recovery. This requires the investigator to identify a calibrator which satisfies the assumptions described earlier. These assumptions may be met more readily *in vitro*, than *in vivo* since the rate-limiting barrier for diffusion *in vitro*, is usually the dialysis membrane. Exceptions to this occur when membrane hydraulic conductivity and transmembrane pressure differences, both hydrostatically and osmotically derived, are different during microdialysis (solute gain) and retrodialysis (solute loss) (25). When using retrodialysis to determine the relative recovery *in vivo*, the proper choice of a calibrator is critical. Often, closely-related structural analogs are tested, and it is important to validate the relationship of the *in vivo* recovery of these calibrators with respect to the analyte of interest. For instance, comparing the recovery of the two molecules in a zero-net flux experiment or examining the *in vivo* loss of each solute from the perfusate can give an indication of calibrator suitability. Moreover, the concentration of the retrodialysis calibrator in the local vicinity of the probe should be considered with regard to changes in saturable metabolism or transport events that may be locally influenced by the presence of the calibrator. The difficulties in finding a suitable reference calibrator have been avoided by some investigators by using tracer quantities of the radiolabelled analyte of interest, and simultaneously measuring the loss of label and the gain of analyte to the perfusate. The availability of radiolabelled analyte often limits the use of this method of determining recovery. Moreover, since this method relies on two different methods of analysis, usually HPLC and liquid scintillation counting, the use of on-line analysis is precluded. However, some laboratories have used the loss of the analyte of interest from the perfusate in pre-experimental and/or post-experimental time periods to allow the determination of recovery of the analyte, thereby allowing on-line analy-

sis, and thus avoiding many of the limitations of the other methods.

Microdialysis studies allow for the collection of a great deal of information without the need for sacrificing a large number of animals. Moreover, the continuous collection of data from a single animal affords one the possibility of identifying intra-animal variability. In more traditional studies of distribution kinetics where a single point is obtained from one animal at one point in time, the investigator is often faced with the difficult task of interpreting population data which encompasses intra- and intersubject variance, with little information concerning the relative contribution of each to the overall variance.

Finally, it should be mentioned that recent advances in the bioanalytical sciences which allow much greater sensitivity may permit the use of much lower dialysis flow rates and thereby enhance relative recovery to the point where the errors inherent in its assessment are acceptably small. For example, recent advances which may allow an interfacing of microdialysis with capillary electrophoresis may permit flow rates in the range of 5 to 25 nl/min, allowing not only a recovery approximating 100% (182) but reasonably short sampling intervals as well. If low-volume, high-recovery methods can be realized without severely compromising analytical sensitivity, a major barrier to more widespread application of this methodology may be breached.

In summary, the use of the microdialysis technique for a variety of pharmacokinetic applications has been reported in animal models and humans, and new applications are being published with greater frequency, particularly with regard to the clinical use of microdialysis. It should be considered that the use of microdialysis sampling in pharmacokinetics is still in the developmental stages, with the continuous evolution of new methods to improve the quantitative aspects of the technique. The technique of microdialysis sampling, when used with careful attention and understanding, will continue to open new doors in the study of pharmacokinetics.

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