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# A survey on quantitative microdialysis: theoretical models and practical implications

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## Contents



## I. Introduction

During the past 10 years, the technique of in vivo microdialysis has been established as one of the major research tools in experimental psychopharmacology and neuropathology. To date, more than 1600 articles have been published. This clearly demonstrates the efficacy of microdialysis in providing valuable information on neurotransmitter release, interactions and metabolism. Similarly, an increasing body of evidence has shown that a proper interpretation of mea-

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sured data is primarily dependent on theoretical knowledge of both qualitative and quantitative processes in microdialysis.

There are two main points in the debate on the usefulness and scientific acceptance of microdialysis: (1) To what extent do the extracellular levels of a neurotransmitter reflect its actual neuronal release? (2) How are the absolute concentrations of a given substance in the ECF quantified?

While criteria for verifying neurotransmitter release are well defined (Herrera-Marschitz et al., 1992), the issue on quantitative microdialysis is still undergoing thorough investigation. Probably the most comprehensive information can be found in a recently published book *Microdialysis in the Neurosciences* in two chapters written by Morrison et al. (1991b) and Benveniste and Hansen (1991). However, these represent only two of several existing theories. In addition, they are not the most common and most frequently used methods available. In fact, Benveniste's approach of using uptake blockers to reach the unaffected extracellular pool did not find any practical consequences.

This paper reviews and summarizes the existing methods of quantitative microdialysis. The methods are divided into 8 groups (A–H) from a historical perspective and applied theoretical principles. The practical implications or limitations of each method are discussed.

## II. Principle of microdialysis and definition of recovery

The principle of dialysis is quite simple: the driving force of molecular movement is diffusion down the concentration gradient existing between two compartments separated by a semi-permeable membrane. For the in vivo model, these compartments represent the extracellular fluid (ECF) of the tissue and the artificial physiological solution inside the microdialysis probe. Diffusion occurs through a membrane at the tip of a probe. Endogenous compounds such as neurotransmitters and metabolites diffuse in, whereas drugs and toxins, if present, diffuse out from the perfusion solution. Fractions are collected and analyzed.

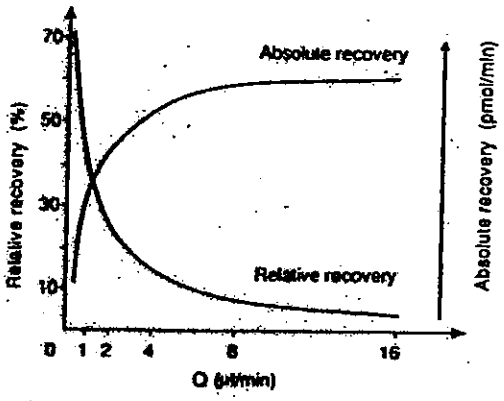


Fig. 1. The effect of flow rate on relative (concentration) recovery and absolute (mass) recovery for a typical microdialysis probe (CMA/10, 4 mm membrane length).

The simplest mathematical description of diffusion is described by Fick's Law. A diffusion model for microdialysis includes a number of additional variables. These include membrane geometry, porosity of the outside compartment and most importantly, the flow rate of perfusion liquid inside the probe. As opposed to equilibrium microdialysis (the term used in drug-binding studies), in vivo microdialysis operates under quasi steady-state conditions. This means that a dynamic equilibrium is established between the concentrations in the two compartments. The ratio between these concentrations is defined as recovery. Recovery expressed in volume concentrations is called relative (or concentration) recovery (Rec) and is inversely dependent on the flow rate (Fig. 1). The higher the flow rate, the more diluted samples will be collected, which means the lower recovery will then be calculated.

Another possibility is to measure dialysis efficiency in mass units removed per time interval (diffusion rate). This is defined as absolute (or mass) recovery. As shown in Fig. 1, mass recovery will increase with flow rate, reaching a plateau at perfusion rates allowing a maximal diffusion flux. Such flow rates are seldom used in practice due to a high risk of 'draining' the compounds from the ECF and thus disturbing the physiological equilibrium. A further increase in the flow rate

reduces the absolute recovery. In this case, the high resistance on the outlet side of the probe causes an increase in hydrostatic pressure and 'sweating' of the membrane. This measurement might have practical importance for optimizing probe design, length of the outlet tubing and molecular cut-off of the membrane.

Only one of the microdialysis models discussed below is built on mathematical description of mass recovery (Ekblom et al., 1992). All the other models quantify relative recovery.

## III. Mathematical models and methods for estimation of extracellular concentrations

The challenge of estimating concentrations of compounds present in the extracellular environment has existed since the first report on microdialysis (Bito et al., 1966). A static approach was used with dialysis bags implanted for several weeks, time enough for equilibration of concentrations on both sides of the membrane.

All the models discussed in this review involve continuous perfusion of the probe which is the key criterion for monitoring extracellular compounds under transient conditions. Only the experimental limitations, both anatomical and analytical, do not allow the use of membranes so long and perfusion rates so low that it will be possible to reach 100% recovery. Therefore, a number of mathematical models and methods for quantifying microdialysis have been proposed. They can be divided into the following: (A) Ungerstedt and Zetterström (1982); (B) Benveniste (1989, 1990); (C) Lindefors and Amberg (1989); (D) Jacobson (1985) and Lerma (1986) (E) Bungay (1990), Morrison (1991), and Merlo Pich (1993); (F) Ekblom (1992); (G) Lönnroth (1987); and (H) 'Reference compounds' (1988–present).

The most explicit equations were derived by groups C and E. Methods used by groups A and D, and F–H are based on purely empirical observations and measurements. The first theories were used to try to define any correlation parameters which allowed the calculation of in vivo levels from in vitro recovery. This approach was soon abandoned, as it became clear that differences in

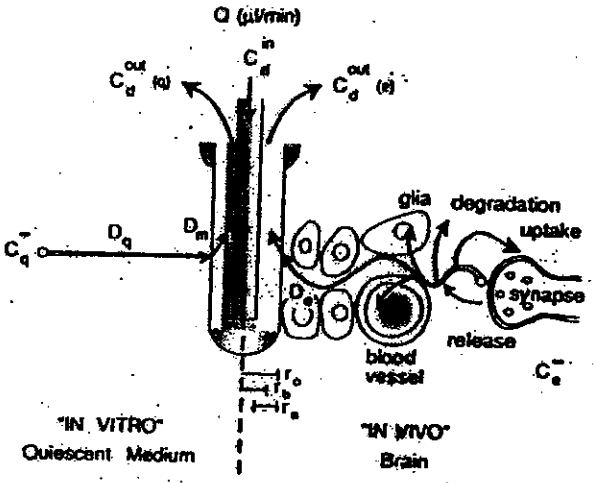


Fig. 2. Schematic illustration of differences between diffusion paths for a hypothetical molecule in vivo (brain) and in vitro (quiescent medium).

diffusion in vitro and in vivo are too large to be able to draw any conclusions. This fact is schematically illustrated in Fig. 2 for a hypothetical diffusion of a molecule in a free solution and in the brain.

In a non-stirred solution, assuming that the temperature and concentration gradients are constant, the diffusion flux is given only by the diffusion coefficients in a solution ($D_q$) and through the membrane ($D_m$). On the contrary, diffusion in the brain is limited to the ECF (about 20% of the total volume) and has a zigzag (tortuous) pattern. The extracellular matrix has a glutinous character due to glycoproteins and other large molecules. Furthermore, the diffusing molecule can be rapidly degraded in the ECF, removed by active processes (both neuronal and glial uptake) or diffuse into a blood vessel. These factors, hindering the diffusion through the ECF, lead to the general assumption that in vivo recovery is always lower than its corresponding in vitro value.

However, recent evidence (see paper by J.B. Justice, Jr. in this issue) suggests that neurotransmitters have greater in vivo recoveries than as those measured in vitro. This implies that the turnover of the transmitter in the ECF plays a crucial role among all factors affecting in vivo recovery. Subsequently, only those methods of quantitative microdialysis which incorporate this

'pulsar-like' behaviour of neurotransmitters in the ECF can be expected to gain broader acceptance in the future.

*III.A. Ungerstedt and Zetterström (1982)*

These authors were the first to recognize the recovery phenomenon and the first to try to calculate in vivo concentrations. The theory is very straightforward, and assumes that the in vitro recovery in a quiescent (non-stirred) medium ($Rec_q$) is the same as the recovery in vivo ($Rec_e$). Thus, by measuring $Rec_q$ and using the same probe and the same flow rate in vivo, the extracellular concentration of a substance $C_e^{\infty}$ can be calculated:

$$Rec_q = \frac{C_q^{out}}{C_q^{\infty}} \quad (1)$$

$$Rec_e = \frac{C_d^{out}}{C_e^{\infty}} \quad (2)$$

$$C_e^{\infty} = \frac{C_d^{out}}{Rec_q} \quad (3)$$

This approximation is very rough since the diffusion of a solute in a free liquid medium is much more rapid than in a porous matrix such as the brain. This is schematically shown in Fig. 2 and discussed in a previous paragraph. The only practical consequence of this model today is that it highlights the importance of measuring in vitro recovieries to check possible disturbances, and the degree and reproducibility of recovery. Reproducibility is significant especially when reusing the probes, when using new batches of probes or when combining microdialysis with complicated and expensive experimental studies.

*III.B. Benveniste (1989, 1990)*

Simultaneous measurements of extracellular $K^+$ and $Ca^{2+}$ by microdialysis and ion-selective microelectrodes has demonstrated that in vivo recoveries are lower than those in vitro, especially for $Ca^{2+}$ (Benveniste et al., 1989). The conclusion from these experiments was that calculations using Eqns. 1 and 3 will lead to the underestimation of concentrations of extracellular transmitters and metabolites. In order to compensate for the hindered diffusion in the brain, Eqn. 3 has been rewritten:

$$C_e^{\infty} = \frac{K \cdot \lambda}{\alpha} \cdot \frac{C_d^{out}}{Rec_q} \quad (4)$$

Here, $\lambda$ is a tortuosity factor correcting for the increased diffusional path, $\alpha$ is the volume fraction of extracellular fluid related to the total brain volume, and factor $K$ expresses the differences in concentrations between the inside of a membrane and the outside medium, both in vivo and in vitro. For flow rates above 2.5 $\mu$l/min, the dialysate concentrations inside the probe approach zero (maximal absolute recovery) and thus factor $K$ can be given the value of 1. For lower flow rates (2 $\mu$l/min), $K$ has been empirically estimated as 0.7. Other constants have empirical values: $\lambda = 1.6$ (range: 1–2) and $\alpha = 0.2$ (Nicholson et al., 1979; Nicholson and Philips, 1981) for non-pathological conditions. The tortuosity factor is defined as (Nicholson and Philips, 1981):

$$\lambda^2 = \frac{D_q}{D_e} \quad (5)$$

where $D_q$ and $D_e$ are the diffusion coefficients in the quiescent medium and in the extracellular space respectively (see Fig. 2). Also, there is a causal relationship (Lipinski, 1990) between $\lambda$ and the extracellular volume fraction $\alpha$:

$$\log\lambda = -0.41 \log \alpha \quad (6)$$

$Rec_q$ should be measured in artificial CSF at pH 7.4 and 37°C. Diffusion coefficients ($D_q$) for most of the endogenous species (cations, anions and neutrals) are about $0.7 \times 10^{-5}$ cm$^2$/s.

Eqn. 4 has been shown to fit well for extracellular calcium measurements, where the calculated ratio $\lambda^2/\alpha$ was 12, the same value as obtained by using microelectrodes (Nicholson and Rice, 1986). However, for neurotransmitters (glutamate, dopamine) this method gave rather disperse and unconvincing values. The concept of correcting concentrations for uptake mechanisms by including uptake blockers produced another approximation, additionally increasing the error of such calculations (Benveniste and Hüttemeier,

1990). Large variations of the tissue parameter $\lambda^2/\alpha$ (1 for $K^+$, 12 for $Ca^{2+}$) resulted in an overweighted influence of this coefficient in Eqn. 4 on the final result.

The most important observation from these measurements was the existence of a drop in concentration of a substance in the vicinity of the membrane (about 1 mm from the surface). It will be shown later (group E) that the profile of this concentration gradient regulates the diffusion flux, i.e., in vivo recovery, and is specific for each substance, or groups of substances.

*III.C. Lindefors and Amberg (1989)*

This model is an attempt to describe in vivo recovery ($Rec_e$) as an explicit function of flow rate, and the diffusion coefficient, length, diameter and porosity of the membrane. The mathematical analysis is based on the theory of diffusion and mass transfer in fluid systems. The resulting equation for $Rec_e$ (Amberg and Lindefors, 1989) is an inverse function of flow rate and corrects for functions describing the time needed to reach steady-state conditions:

$$Rec_e = \frac{4\pi L D_q \alpha}{Q \cdot \lambda^2} \cdot \left[ h(t) + \underbrace{\frac{\alpha}{\lambda^2}(M_1 + M_2)\, g(t)}_{B} \right] \qquad (7)$$

Here $h(t)$ and $g(t)$ are complicated functions of non-dimensional time, defined as $t = t^* \cdot D_e / r_o^2$. $t^*$ is the time needed for establishment of a steady-state concentration profile, i.e., steady-state outflow concentrations in the brain tissue during dialysis (taking 1.5 h or 5400 s as the shortest satisfactory interval). Values $h(t)$ or $g(t)$ can be extrapolated from graphs in the literature (Amberg and Lindefors, 1989). Coefficients $M_1$ and $M_2$ describe the hindering effect of the membrane and can be calculated from probe and membrane geometry, thickness, porosity and diffusion coefficients. Part B in Eqn. 7, including function $g(t)$, describes the reduction of recovery if the flow rate $Q$ is too slow to keep the concentration inside the membrane surface close to zero (see the analogy with factor $K$ in Benveniste's equation). Usually, if $Rec_e$ is less than 0.2 and $Q$ is greater than 2 $\mu$l/min, part B can be neglected. Substituting Eqn. 7 in Eqn. 2, the undisturbed extracellular concentration is calculated as (Lindefors et al., 1989):

$$C_e^{\infty} = \frac{Q \cdot \lambda^2}{4\pi L D_q \alpha} \cdot \frac{C_d^{out}}{h\left(5400 \cdot D_q / r_o^2 \lambda^2\right)} \qquad (8)$$

For actual calculations of extracellular $C_e^{\infty}$, one needs to know the probe dimensions ($L$,$r_o$), diffusion coefficient $D_q$ at 37°C (e.g., Rice et al., 1985), $\lambda = 1.6$, $\alpha = 0.2$ and value $h(t)$. This value normally ranges between 0.23 for $Ca^{2+}$ (Benveniste and Hüttemeier, 1990) and 0.42 for Substance P (Lindefors et al., 1989). Concentration in the microdialysate $C_d^{out}$ is measured experimentally at a given flow rate $Q$.

This model provides theoretical evidence that perfusion during microdialysis leads to a quasi steady-state between concentrations of a solute diffusing through the membrane. Eqn. 8 does not consider chemo-biological differences of molecules in the ECF. For example, the only variable indicating the difference between dopamine and DOPAC is the in vitro diffusion coefficient $D_q$. Thus, in spite of its theoretical value, this model is considered to be too mechanistic.

*III.D. Jacobson (1985) and Lerma (1986)*

Recent computer programs make it quite easy to use non-linear regression analysis of an experimentally measured curve depicting the function of the concentration $C_d^{out}$ on the flow rate $Q$. The curve is analogous to that shown in Fig. 1 for $Rec = f(Q)$. By extrapolating the curve to zero flow, the recovery will attain a value of 1 (or 100%). At this point, the $C_d^{out}$ is equal to the extracellular concentration $C_e^{\infty}$. The exponential dependence of recovery on flow rate $Q$ can be mathematically formulated as (Jacobson et al., 1985):

$$Rec_e = \frac{C_d^{out}}{C_e^{\infty}} = 1 - \exp(-K_m \cdot S / Q) \qquad (9)$$

where $K_m$ is the average mass transfer coefficient, and $S$ is the membrane surface area. This model assumes that $K_m$ is constant and does not vary with $Q$ (in the range 0.5–10 $\mu$l/min), which also means that the concentration profiles out-

side the probe should be constant. Furthermore, the dialysis membrane is supposed to constitute a major diffusion barrier. Although this is not entirely true, this model has found some important applications and correlates well with Lönnroth's model (De Boer et al., 1991; Parsons et al., 1991; Ståhle et al., 1991; Menachery et al., 1992; Parsons and Justice, 1992). Practical calculations are based on measuring several concentrations, $C_d^{out}$, at varying flow rates which will give a corresponding number of equations:

$$C_{dn}^{out} = C_e^{\infty}[1 - \exp(-K_m \cdot S/Q_n)] \quad (10)$$

where $n$ is the number of measurements.

Using a least-squares successive approximation to fit the Eqns. 10 to the experimental data, one can obtain the product $K_m \cdot S$ and the apparent extracellular concentration, $C_e^{\infty}$. Graphical representation of $C_d^{out}$ versus $Q$ gives a slope corresponding to $K_m \cdot S$ and $C_e^{\infty}$ is the intercept at zero flow.

To increase the number of Eqns. 10 and thus to evaluate $K_m$ with the least error, it is also possible to vary the membrane length (area $S$). In practice, this is achieved by implanting two microdialysis probes simultaneously in muscle or adipose tissue. In brain microdialysis, it is possible to perfuse with different calcium concentrations (e.g., 1.2 and 2.4 mM, as in Parsons and Justice, 1992) or alter recovery by chemical lesions (Parsons et al., 1991) and obtain different concentrations ($C_d^{out}$) of a released transmitter. The coefficient, $K_m$, should be constant, unless excessive 'draining' of the tissue occurs.

Aa equation similar to Eqn. 10, which is derived from the kinetics of elimination of a substance from tissue in a 1-compartment model, was published by Lerma et al. (1986):

$$C_d^{out} = C_e^{\infty}[1 - \exp(-k \cdot t)] \quad (11)$$

where $k$ is the rate constant for elimination and $t$ is the dialysis time for a differential of a volume in a dialysis tube defined as:

$$t = v \cdot n/Q = \frac{S \cdot r_0}{2Q} \cdot n \quad (12)$$

where $V$ is the internal volume of the dialysis tube and $n$ is the number of recirculations of volume $V$, where the medium perfuses in a closed loop. Extracellular concentrations were calculated by varying flow rates or recirculating the perfusate $n$ times, and applying a non-linear curve-fit to experimental data. This approach has not attracted much attention probably because it is common to use syringe pumps which do not allow recirculation of the perfusate.

The latest method based on variations of the flow rate is described by Merlo Pich et al. (1993). The perfusion flow is stopped for a fixed period of time and then it is restarted again to remove the concentrated dialysate plug out from the probe. Obviously, the longer the stop-flow interval the higher the concentration in the dialysate will be found. This function can be mathematically described as:

$$m_d^{out} = 2 \cdot C_e^{\infty} \cdot \sqrt{\frac{D_m \cdot T}{\pi}}\, T \quad (13)$$

Here $m_d^{out}$ is the mass of a substance which diffused through a given membrane surface during the stop-flow period $T$. $D_m$ is the diffusion coefficient through the membrane. Measuring $m_d^{out}$ at different stop-flow periods should allow calculation of $C_e^{\infty}$ using the regression analysis. However, is it assumed $D_m$ is the rate limiting step (at least for large molecules such as CRF), thus once estimated in vitro $D_m$ could also be used for in vivo measurements.

*III.E. Bungay (1990) and Morrison (1991)*

This is an expanded version of Jacobson's model in an attempt to further specify the mass transfer coefficient $K_m$ from physical and biochemical processes occurring in vivo. A new variable, an overall probe – external medium permeability $P$ (equivalent to $K_m$), is introduced (Bungay et al., 1990), and defined as:

$$K_m = P = \frac{1}{S(R_d + R_m + R_e)} \quad (14)$$

Substitution in Eqn. 9 will give:

$$Rec_e = \frac{C_d^{out}}{C_e^{\infty}} = 1 - \exp\left[\frac{-1}{Q(R_d + R_m + R_e)}\right] \quad (15)$$

Here, in vivo recovery is expressed as a function

of the flow rate $Q$ and the sum of mass transfer resistances of a dialysate $R_d$, membrane $R_m$ and tissue $R_e$. Parameters $R_d$ and $R_m$ are functions of cannula and membrane dimensions, porosity and diffusion coefficients in the membrane and free medium (see the analogy with coefficients $M_1$, $M_2$ in Lindefors' model, Eqn. 7). Resistance to solute movement in the tissue, $R_e$, is given by:

$$R_e = \frac{(K_0/K_1)\Gamma}{S \cdot D_e \cdot \alpha} \tag{16}$$

where $\Gamma$ is the concentration profile penetration depth defined as:

$$\Gamma = \sqrt{\frac{D_e}{k_e}} \tag{17}$$

$K_0$ and $K_1$ are modified Bessel functions of the second type (tabulated) with argument $r_0/\Gamma$, $k_e$ is the overall rate constant of disappearance of the solute from the extracellular space due to metabolic and exchange processes (see also Fig. 2).

This is the first model which considers active processes in a living body. However, since most of these constants are as yet unknown, the Eqn. 15 has limited application. Some compromise with empirical measurements is thus necessary. For example, the penetration depth $\Gamma$ can be measured experimentally using quantitative autoradiography of labelled substances (Dykstra et al., 1991, 1992). This will allow the calculation of $R_e$ ($D_e$ is calculated from Eqn. 5, and from Eqn. 6). For most of the compounds and probe membranes thus far examined in vivo, the resistances are $R_e \gg R_m \gg R_d$. However, during in vitro measurement in a well-stirred medium, $R_e = 0$, while $R_m$ and $R_d$ have the same values as in vivo (Hsiao et al., 1990; Bungay et al., 1991). Thus, such an experiment could be used for evaluation of $R_d$ and $R_m$ by the same procedure as in Jacobson's model. In practice, this means that, knowing the concentration profile of a substance (drug or neurotransmitter) around the probe and measuring the membrane resistance in vitro, an estimation of in vivo extracellular concentrations could be made from a single measurement.

The most recent extension (Morrison et al., 1991a,b) of Bungay's steady-state theory also includes time-dependent equations describing transient recoveries and tissue concentration profiles. The theory is useful for predicting concentration profiles of exogenously administered compounds, as well as for calculations of the time needed for stabilization of in vivo recovery.

*III.F. Ekblom (1992)*

This model resembles the two previous methods as it is also based on a non-linear regression to zero flow. However, the computer fit is not related to the curve for relative recovery, but to the curve describing the mass recovery (see Fig. 1). The equation is analogous to the Michaelis-Menten equation, rewritten for the function of diffusion flux versus flow rate (Ekblom et al., 1992):

$$\frac{dC_d^{out}}{dt} = J_{max} \cdot \frac{Q}{Q + Q_{1/2}} \tag{18}$$

$J_{max}$ is the maximal diffusion rate and $Q_{\frac{1}{2}}$ is the flow rate at one-half of $J_{max}$. Physically, $Q_{\frac{1}{2}}$ stands for the medium permeability as defined in Bungay's model. Rearranging the Eqn. 18 gives:

$$C_d^{out} = J_{max} \cdot \frac{1}{Q + Q_{1/2}} \tag{19}$$

and for zero flow ($Q = 0$), $C_d^{out}$ must equal $C_e^{\infty}$:

$$C_d^{out} = \frac{J_{max}}{Q_{1/2}} = C_e^{\infty} \tag{20}$$

Parameters $J_{max}$ and $Q_{1/2}$ can be calculated by computer fit to the Eqn. 18 for different flow rates.

It has been shown that this method provides satisfactory data for calculations of extracellular drug (morphine) concentrations in rat striatum. The model was further simplified by incorporating a function of diffusion clearance of a drug which is assumed to equal the tissue permeability. $C_e^{\infty}$ can be calculated directly from the equation:

$$C_d^{out} = \frac{Q_{1/2} \cdot C_e^{\infty}}{Q + Q_{1/2}} \tag{21}$$

The validity of this model for neurotransmitters and endogenous compounds has not yet been tested.

*III.G. Lönnroth (1987)*

Explicitly derived models of in vivo recovery (Amberg and Lindefors, 1989; Benveniste et al., 1989; Bungay et al., 1990) are still dependent on a number of unavailable constants ($D_m$, $D_e$, $k_e$, $K$, etc.) or approximated coefficients, such as $\lambda$ and $\alpha$. Therefore, several purely empirical models for the estimation of in vivo recovery and extracellular concentrations have been developed. The following model is based on a very simple assumption.

If the same compound as in the ECF is present in the perfusion medium, the direction of diffusion flux can be either into or out from the probe, depending on where the higher concentration is present. However, there must be one point where the concentrations are equal, i.e., $C_d^{out} = C_d^{in} = C_e^{\infty}$. At this point of no-net-flux, no diffusion across the membrane occurs since there is no concentration gradient. Recovery, $Rec_e$, can be defined in a more general way (Bungay et al., 1990) as:

$$Rec_e = \frac{C_d^{out} - C_d^{in}}{C_e^{\infty} - C_d^{in}} \qquad (22)$$

A plot of $C_d^{out} - C_d^{in}$ against $C_d^{in}$ should yield a straight line with slope $Rec_e$ and intercept $C_e^{\infty}$ on the abscissa. The optimal curve is calculated by linear regression analysis or, possibly, by Taylor's approximation.

The method has been used in practice for the estimation of extracellular concentrations of glucose (Lönnroth et al., 1987; Bolinder et al., 1989; Henriksson et al., 1990), lactate (Jansson et al., 1990a; Scheller and Kolb, 1991; Kurosawa et al., 1991), glycerol (Jansson et al., 1990b), adenosine (Lönnroth et al., 1989), drugs (Ståhle et al., 1990, 1991a,b; Menachery et al., 1992) and recently for dopamine and DOPAC (Parsons et al., 1991; Parsons and Justice, 1992; Smith et al., 1992; Justice, this issue). Furthermore, it was demonstrated that the method allows for quantitative analysis of extracellular dopamine and DOPAC under transient conditions, such as when provoked by drugs like cocaine, amphetamine and haloperidol (Olson and Justice, 1993). The technique gives excellent results for pharmacokinetic calculations of extracellular drug concentrations and investigations of drug distribution in tissue compartments.

*III.H. 'Reference compounds' (1988–present)*

Several attempts have been made to find an internal marker of in vivo recovery which might serve as a reference for calculations of extracellular concentrations of drugs or endogenous compounds. Three approaches can be recognized:

*III.H.1. Systemically introduced markers.* The use of a compound which distributes equally in all tissue compartments (especially ECF and blood) allows calculations of extracellular volumes. One of the most suitable substances is tritiated water which, if administered intravenously, equilibrates with extravascular body fluids within 1–2 h. According to Eqn. 2, measuring the radioactivity in blood samples and in microdialysates (Alexander et al., 1988; Terasaki et al., 1992; Yokel, 1992) will give a direct value of the recovery in vivo. This recovery can be used for calculations of extracellular concentrations of systemically applied drugs such as theophylline (Sjöberg et al., 1992) or markers of the blood–brain barrier and brain tissue damage (Yokel et al., 1992). Another possibility is to measure, both in vitro and in vivo, the dialysis clearance of labelled water, urea, antipyrine or sucrose. By using a modification of Eqn. 9, it should be possible to calculate interstitial concentrations (Deguchi et al., 1991; Terasaki et al., 1992). Other compounds such as inulin, mannitol, thiosulphate, sulphate and bromide, which are used as extracellular markers in clinical chemistry, could also be useful.

*III.H.2. Locally introduced markers.* This method uses markers added to the perfusion medium. A loss of the compound into the tissue is measured from the outlet of the microdialysis probe. It is assumed that this 'reverse' recovery is the same as the 'normal' ECF → perfusate recovery. Furthermore, it is advisable to use a marker which is chemically and biologically similar to the measured substance. For instance, Larsson (1991) used caffeine as a marker for measuring theophylline in lungs. Similarly, for calculations of the drug AZT, Wong et al. (1992) infused the marker

AZddU which differs from AZT only by one methylene group. Ideally, the marker should be the same substance with a radioactive entity (e.g., $^{14}$C-labelled lactate as used by Kurosawa et al., 1991). Its concentration in the perfusion medium can be roughly estimated by Lönnroth's method. Loss of the radioactivity can be measured together with total chemical concentration. More general markers such as tritiated mannitol in the brain (Fink-Jensen et al., 1991) or ethanol (Hickner et al., 1991) in muscle have also been tested.

*III.H.3. Endogenous markers.* It is hypothesized that 'neutral' endogenous compounds might be used to obtain a reference baseline for changes in all other 'responding' substances. Such data presentation is often used in analytical biochemistry for the quantification of tissue microsamples where the concentration of a substance is related to the total protein content in the sample, rather than to the total volume or mass.

Similarly, in clinical chemistry, immediate levels of compounds analyzed in urine are measured with respect to creatinine content. Such an approach might be also applied in microdialysis. The only question is to find sufficiently inert endogenous markers. In addition to organic molecules, such as creatinine or urea, obvious candidates are some of the inorganic ions usually not present in a common perfusion medium, e.g., $Mg^{2+}$, $PO_4^{3-}$ or $CO_3^{2-}$.

Some practical consequences for the investigator could be a better control of microdialysis experiments, and possibly a better chance of comparing data published by different groups using different probes. A reference compound is definitely desirable for microdialysis in peripheral tissues. The marker should serve as a correction factor for any changes of recovered substances caused only by variations in blood flow and not by cell metabolism itself. This also applies to the brain during any treatment affecting the blood–brain barrier.

## IV. Conclusions

The development of microdialysis has passed its heuristic period to become a technique based on more solid theoretical ground. Besides neurobiologists studying biological mechanisms using microdialysis, chemical engineers and physical chemists have become interested in studying theoretical mechanisms of microdialysis incorporated to biological systems.

However, the empirical position is still overwhelming. The best results have been achieved using the variation of concentrations (point of no-net-flux technique) and by varying flow rate (zero-flow method). Several papers have been published on the use of reference compounds, mostly in pharmacokinetic studies.

An unanswered question is the invariability of in vivo recovery under changed physiological/pathological conditions. Recent results demonstrate that under stimulation or in lesions, in vivo recovery of dopamine is significantly decreased (Justice, 1993). Furthermore, it is higher than the corresponding in vitro recovery. If this observation is confirmed for other neurotransmitters, the recovery theorem will have to be reconsidered as a possible new variable.

Finally, progress in the quantitative estimation of extracellular compounds is direcly dependent on the development of better sampling and analytical tools. For example, an ideal analytical method would be capillary liquid chromatography or electrophoresis, allowing analysis of only a few nanolitres of samples (see paper by A. Ewing in this issue). This, in turn, will permit perfusions at flow rates close to zero, which means almost 100% recovery (Menachery et al., 1992). At this point, no formulae are required.

## V. Summary

The existing methods of quantitative microdialysis are reviewed. The methods are divided into 8 groups, depending on the mathematical models and theoretical principles used to describe convective diffusion in the extracellular space of the brain. Special emphasis is made to describe each method from a historical perspective, showing its main contribution to recent knowledge, as well as its limitations and drawbacks. It is concluded that those methods based

on explicitly derived equations for in vivo recovery are still too approximative and not suitable for routine application. Therefore, empirical models based on varying perfusion flow rates or concentrations of substances in the perfusion solution, found several practical implications. Methods using a reference substance as a marker of in vivo recovery are also discussed. The paper stresses the increasing importance of methods allowing the quantitative evaluation of microdialysis data whenever measuring neurotransmitter release, drug concentrations or pharmacokinetic variables.

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