

Microdialysis- Studying drug distribution and kinetics

How do we measure drugs today ?

Determination of drug levels in body fluids (blood, urine, CSF) is a routine clinical procedure for correlating pharmacological therapy with the manifestation of a disease. These measurements give average information about absorption, metabolism and excretion of drug in the body.

However, the most important determination of the action profile of a drug is its distribution within

the body organs and its time course. For these reasons, a number of samples often has to be collected over a time period and samples from individual tissues (e.g. muscle, liver, tumours) are required. The measurements of drugs in tissue (biopsies, experimental in vivo techniques) are limited by the fact that they do not discriminate between free concentration and drug bound to cell components.

Why Microdialysis?

Before a molecule in the blood can enter a cell in an organ, it must first traverse the extracellular space in the tissue. The same is true in the opposite direction for transport of cell (drug) metabolites into the blood.

Sampling of the extracellular fluid by Microdialysis enables continuous monitoring of intercellular chemical communications. Investigations of the mechanisms of drug actions can thus be performed in a very specific area of a living body. Microdialysis is a very attractive alternative in the field of classical experimental pharmacokinetics for the following reasons:

It gives the true picture of drug distribution and kinetics by:

- Continuous blood sampling without removing liquid.
- Monitoring of the drug time course in different organs, in the same animal.
- Fast time resolution which enables calculations of kinetic parameters.

- Simultaneous measurement of a drug and endogenous compounds affected by a drug.
- The possibility of administering a drug either systemically or locally via the probe.

It is the very convenient way of sampling because:

- The microdialysis probe operates as an artificial blood vessel.
- Samples can be taken continuously from freely moving animals for periods up to several days.
- No behavioural or neurological disturbances are observed with brain implanted animals.

Economical and ethical considerations.

- On average, 5-10 times fewer animal experiments have to be performed for the dose and time profiling of a drug.

How easy is it?

The following examples show the feasibility of Microdialysis in three different situations: sampling of acetaminophen from blood, differences in distribution of caffeine and theophylline within a body and simultaneous determination of a stimulant drug (cocaine) and responding neurotransmitter (dopamine) in the rat brain.

In all cases the microdialysates were injected directly, without any pre-treatment, into the liquid chromatograph.

Fig. 1. The time course of absorption of acetaminophen (APAP) from blood in rat.

At 0 min, rat was given 10 mg/kg APAP i.p. **CMA/10 Microdialysis Probe** with a 4 mm membrane was implanted in the jugular vein and perfused with Ringer solution at a flow rate 2 $\mu\text{l}/\text{min}$, 5 min, fractions were collected and analyzed by HPLC using electrochemical detection.

The peak concentration of APAP in microdialysates occurred between 10 - 15 min, after injection. This corresponds well with data reported for whole blood sampling (3). The half-life of the drug calculated from present data was 15.3 min.

APAP penetrates the blood-brain barrier and has shown to reach peak concentrations in the extracellular space of the brain within 40 - 60 min. after i.p. injection. Perfect time course correlation was observed between Microdialysis and *in vivo* voltammetry, whereas tissue APAP values followed a different pattern (3). Recently, distribution curves of the drug and its sulfate and glucuronide conjugates sampled by Microdialysis were studied in blood and liver as well (4).

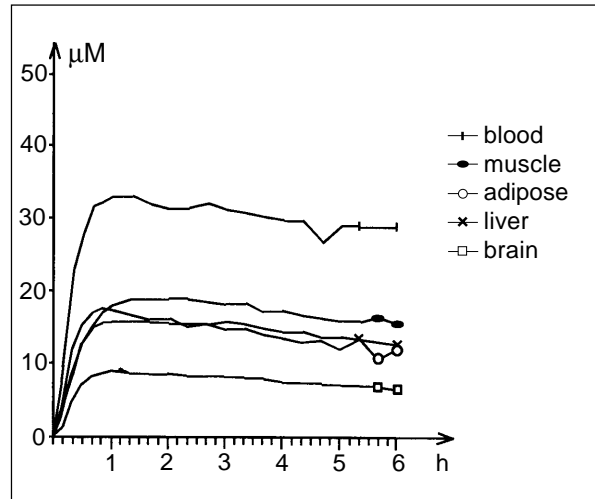
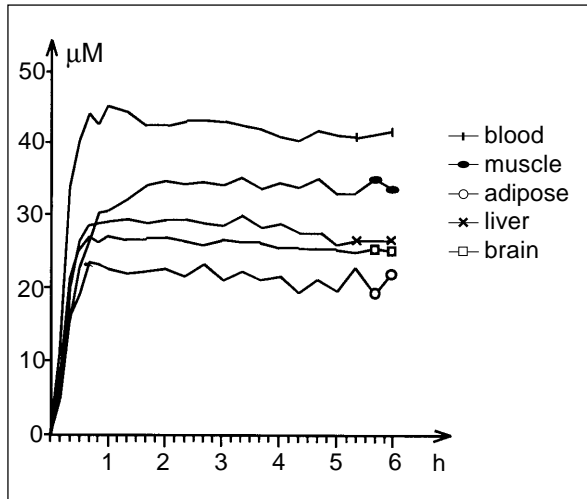
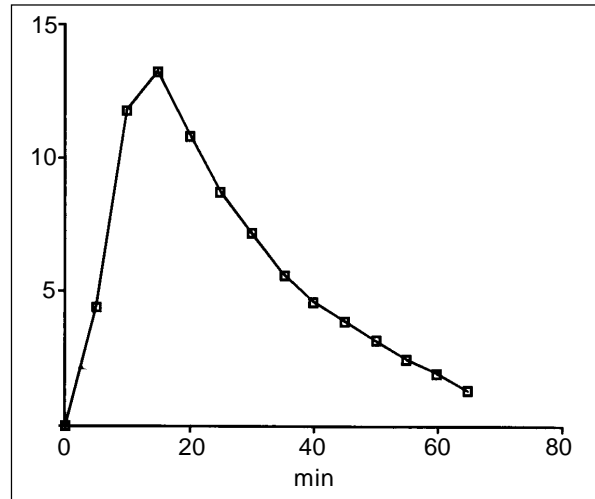


Fig. 2. Distribution of caffeine and theophylline in the rat (1)

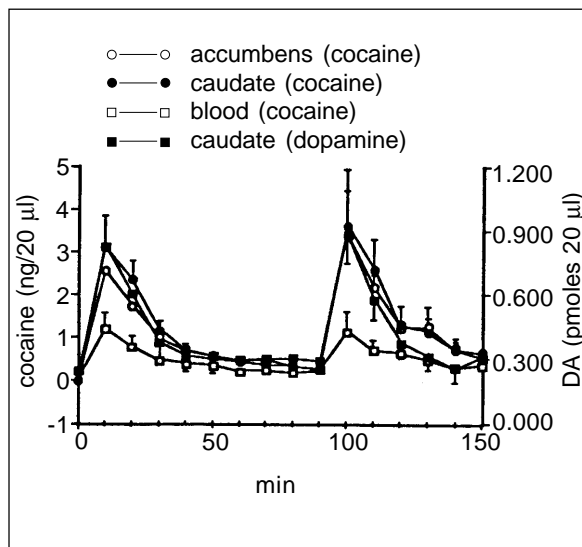
CMA/10 Microdialysis Probes (3 mm membrane length) were implanted in the striatum, jugular vein, fat tissue (neck), liver and muscle of a rat. Probes were perfused with Ringer at a rate of 1 $\mu\text{l}/\text{min}$. 10 min fractions were collected during the first hour after s.c. injection of caffeine and theophylline (each 20 mg/kg), followed by 20 min. sampling intervals. Samples were analyzed by reverse phase HPLC with UV detection.

Comparing the levels of caffeine and theophylline, it is apparent that the free concentrations of caffeine are higher than those of theophylline. The reason is most probably the high protein binding of theophylline (up to 60%). Similarly, the considerably lower concentrations of theophylline in the brain indicate its poor penetration through the blood-brain barrier. This can partly explain its lower effects on the central nervous system as compared to caffeine.

Fig. 3. Distribution and pharmacokinetic profile of cocaine followed by dopamine overflow in rat striatum (2).

CMA/10 Microdialysis Probes (2 and 4 mm membrane length) were implanted in the brain nuclei and the jugular vein of a rat and perfused with Ringer at 2 μ l/min. Two intravenous (via the femoral vein) injections of 1.5 mg/kg cocaine were given 90 min apart. Fractions were collected every 10 min and assayed for cocaine and dopamine (brain) by reverse phase HPLC:

As can be seen, cocaine and dopamine concentrations followed the same profile for both injections. They reached a maximum within the first 10 min after administration. The half-lives of cocaine in the regions under study were estimated to be between 20 - 30 min. Furthermore, the data indicate that the inhibitory effect of cocaine on the DA uptake system is fast and reversible. In addition, the mechanisms regulating extracellular DA levels are quite efficient in restoring the DA content back to the original baseline levels.



What has been published about the use of Microdialysis in pharmacokinetics and drug analysis?

1 Ståhle L., Segersvärd S., and Ungerstedt U. (1991) Drug distribution studies with microdialysis.

II. Caffeine and theophylline in blood, brain and other tissues in rats. *Life Sci.* 49, 1843-1852.

2 Hurd Y., Kehr J., and Ungerstedt U. (1988) In vivo microdialysis as a technique to monitor drug transport: correlation of extracellular cocaine levels and dopamine overflow in the rat brain. *J. Neurochem.* 51, 1314-1316.

3 Sabol K.E., and Freed C.R. (1988) Brain acetaminophen measurement by in vivo dialysis, in vivo electrochemistry and tissue assay: a study of the dialysis technique in the rat. *J. Neurosci. Meth.* 24, 163-168.

4 Scott D.O., Sorensen L.R., and Lunte C.E. (1990) In vivo microdialysis coupled to liquid chromatography for the study of acetaminophen metabolism. *J. Chromatogr.* 506, 461-469.

5 Caprioli R.M., and Lin S.N. (1990) On-line analysis of penicillin blood levels in the live rat by combined microdialysis/fast-atom bombardment mass spectrometry. *Proc. Natl. Acad. Sci. USA*, 1, 240-243.

6 Ben-Nun J., Cooper R.L., S.J., and Constable I.J. (1988) A new technique for in vivo intraocular pharmacokinetic measurements. *Arch. Ophthalmol.* 106, 254-259.

7 Menacherry S.D. and Justice J.B. (1990) In vivo microdialysis and thermospray tandem mass spectrometry of the dopamine uptake blocker 1-2-Bis (4-fluorophenyl) methoxy ethyl-4-3-(3-phenylpropyl)piperazin (GBR 12909). *Anal Chem.* 62, 597-601

8 Quan N., and Blettis C.M. (1989) Microdialysis: a system for localized drug delivery into the brain. *Brain Res. Bull.* 22, 621-625.

9 Dubey R.K., Mc Allister C.B., Inoue M., and Wilkinson G.R. (1989) Plasma binding and transport of diazepam across the blood-brain barrier. *J. Clin. Invest.* 84, 1155-1159.

10 Ferraro T.N., Weyers P., Carozza D.P., and Vogel W.H. (1990) Continuous monitoring of brain ethanol levels by intracerebral microdialysis. *Alcohol.* 7, 129-132.

11 More than 400 other articles where Microdialysis was used to monitor extracellular levels of endogenous compounds (neurotransmitters and neuromodulators, nutrients and metabolites, ions) in physiology, behaviour, neuropathology, psychopharmacology, are indexed in a separate book (Library of Microdialysis, Bibliography, 1992, 1993, CMA/Microdialysis).

If you require further details on Microdialysis procedures, HPLC analysis, instrumentation or bibliography, please do not hesitate to contact:

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