MINIREVIEW: MICRODIALYSIS OF THE BLOOD OUTFLOWING FROM THE BRAIN

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In past several years the *in vivo* blood microdialysis technique has been widely used for a variety of pharmacological and physiological applications to study, monitor and analyze endogenous substances, such as neurohormones, and exogenous substances such as therapeutic drugs and their metabolites. The technique is being desctribed in detail and discussed, in which microdialysis probes were implanted into the jugular vein, blood was flowing freely around the dialysis membrane. The probe was perfused at a very low flow rate (~ 1-2 μL/min)with the solution resembling closely the composition of body fluid. In this laboratory (Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz) the technique of *in vivo* blood minidialysis was worked out in small laboratory animals (rat, guinea-pig, hamster) and used to demonstrate that neurohypophysial hormones can be released into the blood outflowing from the region of the sella turcica and blood dialysate from the femoral vein.

Key words: Microdialysis - Cavernous sinus – Brain blood – Small animals - Minireview

Historical introduction

The dialysis has been used to separate low-molecular compounds from macromolecular systemic fluids or tissue extracts. The dialysis rate is directly proportional to the difference of concentrations on both sides of the dialysis membrane, the surface area of the membrane and the permeability coefficient dependent primarily on the temperature and character of the membrane-dialysed compound system. Purification of the blood from toxic substances outside the organism was initiated by a German doctor from Giesen, George Hass in 1914, who proposed to use heparin as a compound inhibiting blood coagulation, because the blood clotted outside the organism. The first to apply successfully the extrasystemic dialysis was a Dutch physician Willem Kolff in 1943. He developed a prototype of an artificial kidney using semi-permeable membranes. His invention aroused great intrest and since that time artificial kidney has been used in clinical practice. The first devices used for dialysis had cellulose dialysis membranes. New dialysing membranes in capillary form made of synthetic materials such as cuprophan, polyether sulfone and polyarylonitryle are used.

In 1964, Hoesli and Monnier reported the presence of delta sleep - inducing peptide in blood dialysates. They carried out dialysis of venous blood outflowing from rabbit brain during electrical stimulation of the thalamic sleep area. The hemodialysate thus obtained proved to induce sleep in recipients of the same species when injected intravenously (MONNIER AND HOESLI 1965; HOESLI et al., 1965; SCHOENBERGER et al. 1972; MONNIER et al. 1972).

Application of microdialysis in vivo in pharmacokinetic studies and drugs metabolism

Within the last two decades microdialysis was developed and it has become a very useful technique for

in vivo pharmacokinetic monitoring and metabolism studies in almost every organ or tissue of the body e.g.: blood (Tsai et al. 2000; Graumlich et al. 2000; Loenroth et al. 1989), liver (Van Belle et al. 1995), lung (Larsson 1991), kidney (Baranowski and Westenfelder 1994; Millat and Siragy 2000), adipose tissue (Arner et al. 1988; Loenroth et al. 1987; Partillo et al. 2000), muscle (Palsmeier and Lunte 1994), bile (Tsai et al. 1999a), brain (Meyerson et al.1990; Hamani 1997). Most *in vivo* microdialysis sampling involves implanting a short dialysis fiber at the sampling site. The fiber is an artificial blood vessel system which can be placed in the extracellular space of various tissues in order to examine these tissues *in situ*. A microdialysis probe is continuously perfused with the solution in composition similar to body fluid at the site at very low flow rate (~ 1-2 μl/min). Molecules are collected from the tissue by the device and their true interstitial concentration can be estimated. Metabolically - active molecules can be delivered to the interstitial space through the microdialysis probe and their effect on the tissue can be investigated locally without producing generalized effects. It is also possible to study local tissue blood flow with microdialysis by adding a flow marker to the microdialysis solvent (Amer 1999). Microdialysis probes for blood dialysis were implanted into the jugular vein, blood was flowing freely around the dialysis membrane. The *in vivo* microdialysis technique allows simultaneous monitoring of endogenous substances and drugs in blood and extracellular fluid (Stahle et al. 1991; Stahle 1991). Using this, Arner et al. (1988) monitored continuously glycerol levels in venous blood and subcutaneous adipose tissue of intact rats. This study illustrates that this technique offers new and unique possibilities for *in vivo* lypolysis studies in intact animals. The *in vivo* technique microdialysis is also tool to investigate delivery of a drug to target tissue and its metabolism by that tissue (Tsai et al. 1999b). Palsmeier and Lunte (1994) utilized microdialysis to investigate the pharmacokinetics and metabolism of the drug SR 4233 (3-amino-1,2,4-benzotriazine-1,4-di-N-oxide, a representative of a new class of bioreductive antineoplastic agents) in blood and in skeletal muscle. Similarly, Nakashima et al. (1996) studied pharmacokinetics and metabolism of drugs in blood and brain. In recent years, microdialysis began to be used in human beings (O'Connell et al. 1996). Brain microdialysis has been performed in patients in the operating room during brain surgery, or in patients in intensive care units (Portillo et al. 2000; Elmquist and Sawchuk 1997). Intravenous microdialysis has been used in very few subjects (Stahle 1991; Paez and Hernander 1996). This technique has also many potential clinical applications for endogenous compounds such as glucose, monoamines and peptides, in pharmacological or pathological conditions or during the *iv* drug administration through the same probe. Venous microdialysis would also allow to monitor the presence of toxins or the levels of prescribed drugs.

It was shown, that recovery of the substance from blood was larger than from other tissues (Larsson 1991); Stahle 1991). Stjernstrom et al. (1993) examined recoveries *in vivo* of small molecules (lactate, hypoxanthine, inosite, adenosine, glucose, creatinine, urea) in patients during intravenous microdialysis and reported to than to be close to 100 %. It is suggested that these high values are due to the relatively large surface area of the probe membranes and the high rate of mass transport in blood compared with that extracellular fluids.

Application of blood microdialysis in vivo for monitoring neuropeptides and neurotransmitters release

Simultaneous microdialysis in blood and distinct brain regions offers the advantage of comparing release patterns of neuropeptides into different compartments directly using the same approach. The nuclear regions of the supraoptical nucleus (SON) and paraventricular nucleus (PVN) are known to respond to direct osmotic stimulation, by increasing vasopressin (AVP) and oxytocin (OXY) release into the extracellular compartment and the blood. Direct stimulation of the SON by microdialysing hypertonic artificial cerebrospinal fluid increased peripheral OXY and AVP release into the blood as well as intranuclear release (Neumann et al. 1993; Ludwig et al. 1994a). The time course of the responses in central and peripheral peptide secretion were different. Immediately after osmotic stimuli, there was an increase in blood dialysate peptide levels. The plasma peptide response was not accompanied by simultaneous increase in peptide release into the SON. In fact, the central response was significantly delayed,

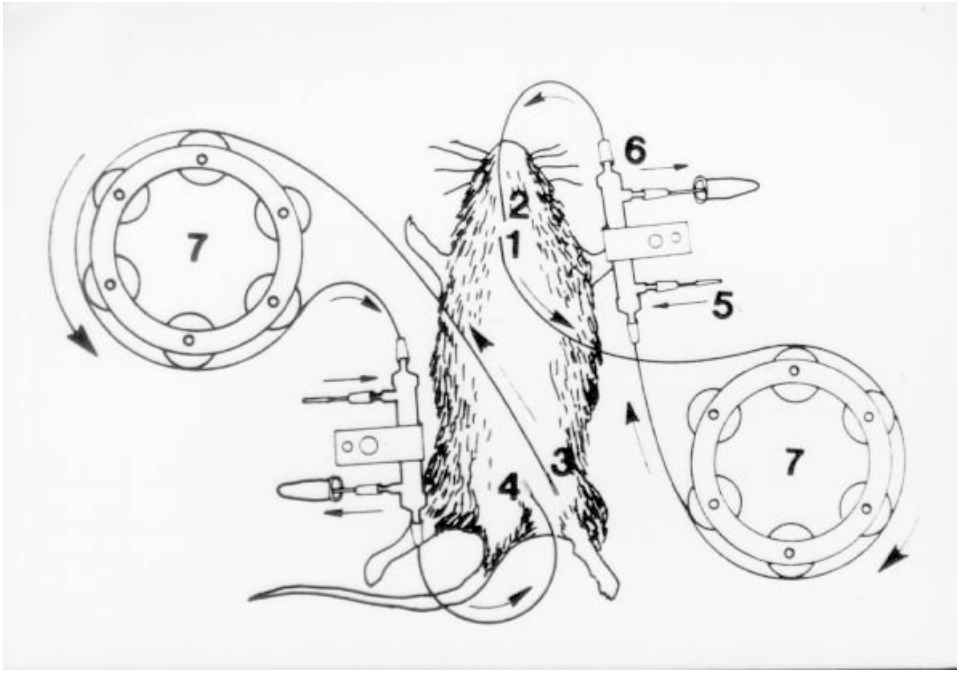

Fig. 1 Dialysis of venous blood outflowing from the cavernous sinus vicinity and from the femoral vein in rats. 1- cannula inserted into the cavernous sinus end of the internal maxillary vein. 2- cannula inserted into the heart end of the internal maxillary vein. 3- cannula inserted into the peripheral end of the femoral vein. 4- cannula inserted into the central end of the femoral vein. 5- inflow tube for filing minidialyser housing. 6- outflow tube for collecting dialysing medium from the minidialyser housing. 7- peristaltic pump.

occurring at the time when plasma AVP and OXY had returned to basal levels (Neumann et al. 1993; Ludwig et al. 1994b; Wojtak et al. 1996).

The technique of microdialysis in blood *in vivo* has been used to monitor and analyze endogenous substances such as neurotransmitters. Microdialysis probe allowed continuous monitoring of free 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid in blood (Rada et al. 1999) as well as changes in extracellular gamma aminobutiric acid (GABA) from a human brain and plasma dialysates (Castejon et al. 1999). Simultaneous dialysis in brain and blood allowed elevation of the different effects antidepressant drug on serotonin and 5-hydroxyindoleacetic acid level at the two sites (Paez and Hernandez 1996).

Dialysis of venous blood outflowing from the cavernous sinus

Euro-Sep Ltd (Polish Scottish joint venture), whose joint owner is the Institute of Biocybernetics and Biomedical Engineering of the Polish Academy of Sciences in Warsaw, in co-operation with the Department of Physiology, have designed and produced minidialysers used for blood dialysis in small laboratory animals (rat, guinea-pig, hamster). In our experiment, samples of dialysates of venous blood outflowing from the vicinity of the cavernous sinus (hypothalamus, pituitary) and, for comparison, from the femoral vein were collected in anesthesized rats (Fig.1).

According to Page (1982) blood enters the hypophysis and hypothalamus through 3 branches of the internal carotid artery: inferior, middle and superior hypophyseal arteries. Blood enters the neural lobe through inferior hypophyseal arteries. It drains from the neural and anterior lobes through the systems of inferior and lateral hypophyseal veins to the cavernous sinus. From the neural and anterior pituitary lobe, blood drains through the systems of lateral hypophysial veins to the cavernous sinus. Blood outflow from the cavernous sinus takes place through the internal carotid artery.

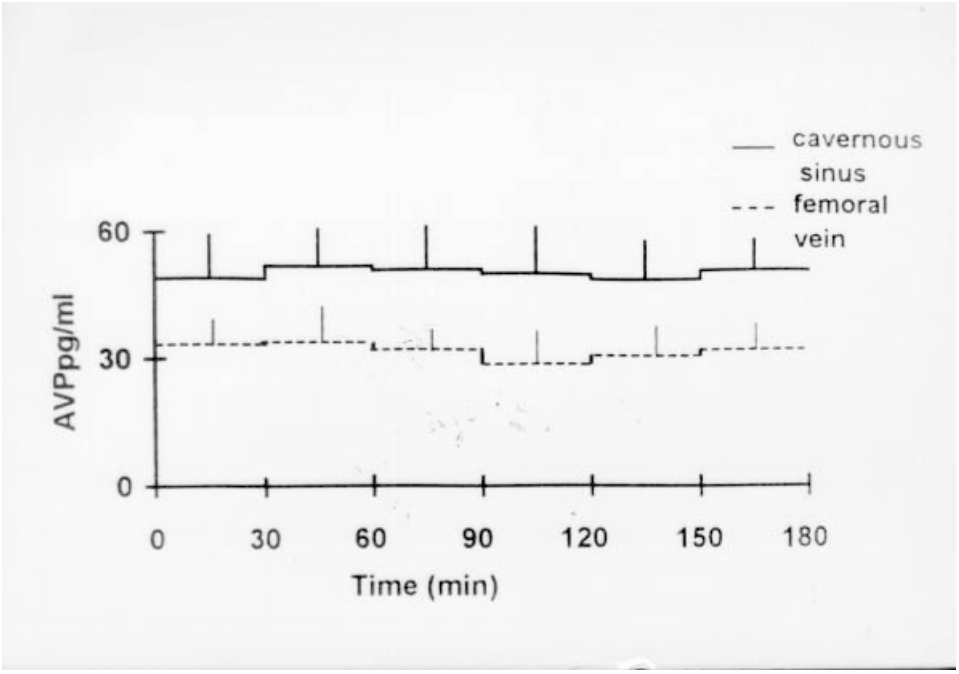

Fig. 2 Arginine vasopressin (AVP) contents in six 30-min blood dialysates collected simultaneously from the the cavernous sinus and from the femoral vein (Values are expressed as means ± SE, P< 0,05; n= 10).

A minidialyser consists of 270 active capillary tubes, each 59 mm long, constituting the total area of 100 cm^2. The dialysing membrane of the capillary tube allowing molecules of molecular mass 20 kDa cutoff value to pass, is made of regenerate cellulose (Cuprophan ®). The inner diameter of the capillary tube is 0.2 mm and the wall is 8 mm thick. Filtration quantity equals 5.5 ml/h/torr. Its casing is made of plastic (trade name Lustran-San ®). It has two tips for Luer's needles for connecting on one side through a cannula with a vein and on the other side with a peristaltic pump. At the side of the minidialyser there are tips for Luer's needles for the exchange of the dialysing fluid. The volume of the dialysed fluid is 0.8 ml and the volume of the dialysing fluid-1.7 ml (Tab. I). Minidialysers were tested *in vitro* and *in vivo* (Goraca and Traczyk 1997).

In order to obtain blood dialysate samples from the vicinity of the pituitary, one polyethylene cannula was inserted into the heart end of the internal maxillary vein and the second cannula into the maxillary vein in the vicinity of cavernous sinus of the sella turcica. Blood was drawn from the region of the sella turcica through a polyethylene cannula (and a tube) to the minidialyser by means of the peristaltic pump. It was then returned to the organism through the cannula inserted into the heart end of the maxillary vein. At the beginning of the experiments, 2 ml of Lock solution with heparin (4000 U/ml) was injected into the internal maxillary vein. Before refilling the minidialyser with dialysing fluid, its cover was rinsed with Mc Ilwain-Rodnight solution. The rate of blood flow through the minidialyser was 0.25 ml/min and the pressure at which blood was dialysed was 10 kPa.

The whole amount of dialysing fluid was exchanged every 30 min for 3 hrs by draining it directly into a test tube. The concentration of vasopressin was determined in dialysates using radioimmunoassay. It was demonstrated that vasopressin concentration was permanently higher in the blood dialysates outflowing from the vicinity of the cavernous sinus in comparison with blood dialysates from the femoral vein. Vasopressin content did not change in the course of dialysis (Fig. 2), whereas AVP content in blood samples withdrawn from the region of the cavernous sinus through the cannula inserted into the internal carotid artery increased during the course of the experiment (Lipinska et al. 1996; Goraca et al. 1996).

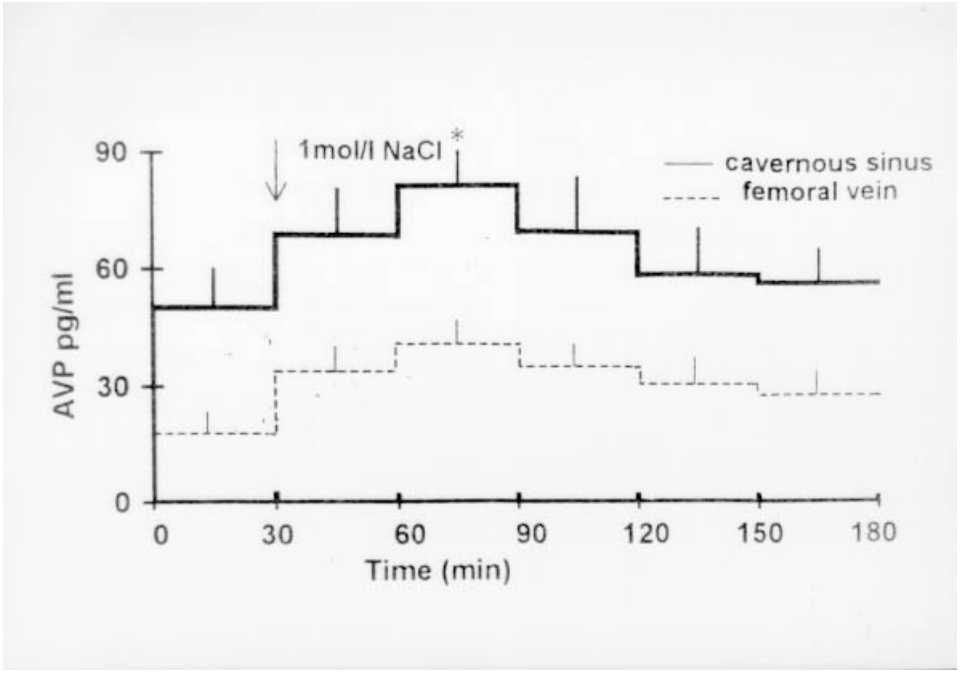

Fig.3 Arginine vasopressin (AVP) contents in six 30-min blood dialysates collected simultaneously from the region of the cavernous sinus and from the femoral vein before, during and after infusion hypertonic saline into the internal carotid artery (Values are expressed as means ± SE, *P< 0,05 vs. sample I (0-30 min dialysis); n= 10).

Osmotic stimulation caused an increase in vasopressin concentration in the blood dialysate samples from the cavernous sinus and from the femoral vein (GORĄCA 1998) (Fig. 3).

The decrease in blood volume consequent to blood sampling causes an increase in vasopressin concentration in blood (Szczepanska-Sadowska et al. 1983; Schreihofer et al. 1994). Coombes and Robinson (1993) collecting serial blood samples from the femoral and jugular vein demonstrated that both basal and osmotic stimulated concentrations were higher in jugular "central" blood samples as compared with the simultaneously withdrawn femoral vein "peripheral" and "central " OXY concentrations. Also other authors showed that vasopressin concentration was higher in the cerebrospinal fluid than in the peripheral blood (Simon-Oppermann et al. 1983).

The previously conducted studies concerning the release of neurohormones to the blood involved collecting the blood from the jugular vessels after decapitation of the animal and determination of the whole blood content of hormones (Zerbe and Palkovits 1984), or determination of hormone content in blood samples (Simon-Oppermann et al. 1983). Collecting blood samples at certain intervals (Ota et al. 1994; Ludwig et al. 1995) makes it possible to observe changes in hormonal release in time.

The microdialysis/or minidialysis technique in blood provides only an average over the neurohormone concentration in limited time, any sharp changes in neuropeptide release are blunted. An adventage of this methods is that it are non-invasive and may be used for continuous monitoring of neurohormonal release and drug pharmacokinetics.

Conventional pharmacokinetics and drug metabolism studies require discontinuous blood sampling over a long period of time. Repeated blood sampling is associated with technical problems (e.g. stressful character of experiment, blockade of catheters, suspension procedures) and with the possibility of producing homeostatic imbalances resulting from loss of large volumes of blood. Reducing the total circulating blood volume may result in a homeostatic imbalance, particulary in small animals which have small total blood volume.

Microdialysis/or minidialysis of blood has several advantages compared with the chemical analysis of whole blood. First, it is not necessery to remove

Table I. Membrane charakteristics of the minidialyser used for the blood dialysis

PARAMETERS	
Ultrafiltration coefficient (ml/h/mm Hg)	5.5
Inulin clearance in vitro (ml/min)	0.52
Molecular cut off (kDa)	20
Active surface (m^2)	0.1
Lumen/wall thickness	200/0.8
Number of capillaries	270
Lenght of capillary (mm)	59
Inner diameter of capillary (mm)	0.2
Priming volume: blood (ml)	0.8
Primaring volume: dialysate	1.7
Membrane material	Cuprophan Ñ
Housing material	Lustran-San Ñ
Ploting compound	Polyurethane
Makximal workin pressure (tor)	500
Net weight (g)	8

Innulin clearance was determined *in vitro* at Ringer-Lock solution and inulin flow rate of 0.45 ml/min.

blood. Furthermore, microdialysis completely avoids the problems with alterations of the blood volume when drawing blood from a small animal such as a rat. Second, it is possible to monitor continuously the extracellular chemical changes without disturbing the animal. Third, there is no need to purify the microdialysates prior to assaying neurohormone contents in the samples, a procedure which is time-consuming and also results in a loss of peptides. As the size of the pores of membrane excludes the proteins, also the enzymatic action is eliminated. Thus, using the technique of minidialysis allows to avoid problems associated with taking blood samples.

In conclusion, the technique of *in vivo* microdialysis/or minidialysis 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 underestimates the importance of microdialysis, primarily in providing the 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.

The technique of *in vivo* microdialysis has become one of the major research tools in physiological and pharmacological studies for monitoring biological active substances such as: hormones and their metabolites. Concentration changes in biologically active substances can be continuously monitored for periods of hours or even days without withdrawing blood samples.

Blood sampling in small laboratory animals causes posthemorrhagic shock and leads to disturbances in neurohormone release from the posterior pituitary lobe into the blood. Microdialysis technique also reduces the total number of animals required in experimental work.

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