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Microdialysis of the brain structures: application in behavioral research on vasopressin and oxytocin

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Minireview

Abstract. *In vivo* microdialysis allows sampling of brain regions in conscious, freely moving animals. Moreover, the *in vivo* microdialysis allows to administer drugs directly into specific brain areas. Both are useful in behavioral studies. The subject of this review is the methodology of brain microdialysis, that is construction of the probe, effect of temperature, composition of the perfusion medium, perfusion flow rate, characteristics of the membrane material and the role of the diffusion coefficient. Other techniques of the study of *in vivo* release, alternative for microdialysis, are described. Advantages and disadvantages of acute and chronic microdialysis are discussed. Chronic microdialysis is especially needed in behavioral studies. Finally, the examples of application of microdialysis in behavioral studies of vasopressin (AVP) and oxytocin (OXT) and our own experience in these studies are described.

Key words: microdialysis of the brain, methodology, acute microdialysis, chronic microdialysis, behavioral studies, vasopressin, oxytocin

Microdialysis offers a way to remove chemical substances from the extracellular fluid of the body without removing the liquid and to introduce substances without injecting fluid. The principle of microdialysis is based on the diffusion of molecules through small-diameter pores of a semipermeable membrane tubing connected to a probe that is stereotaxically implanted into a defined brain area. The probe is connected to a perfusion pump and perfused with a liquid, which equilibrates with the fluid outside the tube by diffusion in both directions. A quantitative analysis of substances in the fraction-collected microdialysates reflects their temporal releasing pattern in the extracellular fluid. Microdialysis also offers the possibility to deliver the substance into the specified brain structure. The substance should be dissolved in the solution perfusing the probe, from which it diffuses into the extracellular fluid in the vicinity of the microdialysis probe (retrodialysis) (Fig. 1). The method has been rapidly adopted in the neuroscienes. In Poland microdialysis was adopted for research at the Medical University of Lodz (Orlowska-Majdak et al. 2003b), Medical Research Center of Polish Academy of Sciences, Warsaw (Hilgier et al. 2003, Łazarewicz et al. 2003), the Institute of Pharmacology of the Polish Academy of Sciences, Krakow (Pietraszek et al. 2002) and at the Medical University of Silesia, Zabrze (Nowak et al. 2002).

Bito and coworkers (1966) were apparently the first to do *in vivo* sampling via dialysis. They implanted dextran – saline filled sacks in both the cerebral hemisphere and subcutaneously in the neck of dogs. The membranes were left implanted for 10 weeks. They were not actively perfused but were left to equilibrate statically. Upon surgical removal of the sacs, Bito et al. analyzed their content of amino acids and electrolytes. Delgado et al. (1972) were the first to perfuse actively the dialysis membrane *in vivo*, and thus were the first to practice the technique, which has since become known as "microdialysis". Delgado's group constructed a "dialytrode" permitting chemical collection and infusion. The device consisted of a pair of parallel cannulae, applied by Gaddum as a push-pull cannula, with dialysis bag cemented to the tip of the cannula. After having implanted their probes in the caudate or amygdala of 10 rhesus monkeys, the group conducted a variety of experiments.

MICRODIALYSIS PROBE

A microdialysis probe typically consists of a cylindrical dialysis membrane, connected with in- and outlet tubes, which is constantly perfused on the inside while the outside is in direct contact with the medium of interest. Two main types of microdialysis probes exist: a) a

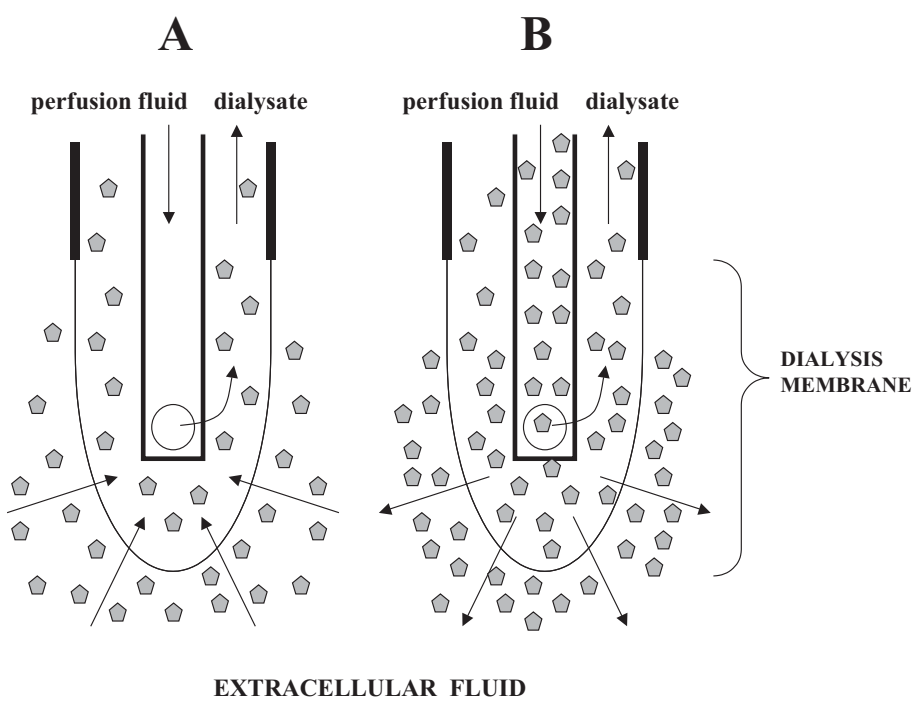

Fig. 1. Directions of substance molecules migration through the dialysis membrane during microdialysis (A) and retrodialysis (B).

horizontal probe where dialysis membrane is connected with in- and outlet tubes in a serial arrangement; and b) vertical probe with in- and outlet tubes positioned parallelly in the form of a loop – U-shaped probe, side by side – I-shaped probe and a concentric probe (Benveniste and Hansen 1991, de Lange et al. 1997b). The construction and dimensions of the probe are important for the *in vivo* tissue reactions and effectiveness of the dialyzing process. The traumatic damage of the brain tissue is the highest after implantation of the U-shaped probe (Horn and Engelmann 2001). The vertical probe with a concentric design is most commonly used. Today, several companies offer commercial microdialysis probes but some investigators make their own probes (Horn and Engelmann 2001). Landgraf et al. (1992) constructed and used a special triple microdialysis probe. It consisted, in addition to the conventional U-shaped probe of a stainless steel cannula for infusion, glued to the two dialysis cannulae. We have had good experience with a vertical concentric probe made by CMA/Microdialysis AB, Stockholm, Sweden (Fig. 2).

In the last two decades the development of combined electrophysiological/neurochemical methods has been observed. Ludvig et al. (1994) described a combination of single-cell recording and intracerebral microdialysis in freely behaving rats. He used a specially constructed electrode/probe assembly. In the 90's, Obrenovitch et al. developed a microdialysis probe, with a miniature electrode inserted in it, which allowed the monitoring of both electroencephalogram (EEG) and direct current (DC) potential at the dialysis site and avoided tissue injury problems due to implantation of separate recording electrodes (Obrenovitch et al. 1993a, 1994). This method proved very useful for correlating electrophysiological changes with neurotransmitter effluxes associated with epileptic seizures (Millan et al. 1991), cerebral ischaemia (Obrenovitch et al. 1993b) and cortical spreading depression (Obrenovitch and Zilkha 1995).

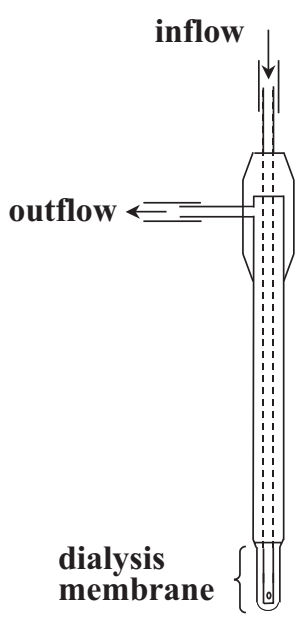

Fig. 2. Design of the commercial concentric probes used in our microdialysis and retrodialysis studies in rabbits.

In the last years the methods for simultaneous multiple intracerebral implantations of microdialysis probes have been described. Due to technical difficulties only a few studies have been published using this approach. Traczyk et al. (1997), Orlowska-Majdak et al. (2001b, 2003a,b), Kołodziejski et al. (2001) implanted two microdialysis probes simultaneously into the hippocampus and caudate nucleus in rabbit, Moor et al. (1994) implanted also two ones into the hippocampus and into the septum and Westerink et al. (1996) into the ventral tegmental area and nucleus accumbens in rat. Hernandez and Hoebel (1989,1995) and Parada et al. (1998) implanted three microdialysis probes into the prefrontal cortex, nucleus accumbens and into the striatum in rat. A special miniaturized, concentric, microdialysis probe was constructed and described for simultaneous, multiple brain microdialysis procedures in rats (Hernandez et al. 1986).

A simultaneous microdialysis of blood and brain structures offers a very useful tool to monitor and compare peripheral and central phenomena. Such a procedure requires implantation of two microdialysis probes: one into the brain structure and the other into the blood vessel. This allows to monitor e.g., concentrations of endogenous (Neumann et al. 1993) or exogenous (Tsai and Liang 2001) substances simultaneously in both compartments.

Brain microdialysis may be combined with microdialysis of the peripheral tissue, e.g., subcutaneous adipose tissue (Lonjon et al. 2001) and with blood and simultaneously with bile microdialysis (Tsai 2001). By applying the microdialysis technique for a biliary excretion study, the number of animals could be substantially reduced as the technique involves a sampling procedure, which does not incur large body fluid losses and, therefore, does not disturb blood homeostasis (Tsai 2001).

There are many companies offering the microdialysis probes and complete equipment for microdialysis (e.g., Applied Neuroscience, CMA/Microdialysis, BASi Bioanalytical Systems).

FACTORS AFFECTING EFFECTIVENESS OF MICRODIALYSIS

The probe implanted into the brain tissue is constantly perfused with a solution, and molecules of endogenous or exogenous agent diffuse across the membrane down the concentration gradient. For most agents equilibrium between interstitium and the perfusion medium is incomplete, therefore the microdialysis probe has to be calibrated in the assumed experimental conditions. The term "relative recovery" and "absolute recovery" characterizes effectiveness of microdialysis. When the concentration of the substance is greater in the medium surrounding the probe than in the perfusion medium and the removal of the substance from the surrounding space occurs, the relation between the concentration of a substance in the perfusion medium to its concentration in the space surrounding the probe is called the relative recovery and is expressed in percentages. The term absolute recovery refers to the total amount of substance removed by the perfusion medium during a defined time period.

Effectiveness of microdialysis expressed as relative recovery depends on:

Temperature

Recovery at body temperature of 37°C is considerably greater than at 22°C (Benveniste 1989). Experiments performed by de Lange et al. (1997a) indicate that perfusate temperature may be especially important in pathological circumstances when periprobe tissue loses its capability to compensate temperature effects. Therefore, it is recommended to perform all microdialysis experiments *in vivo* and *in vitro* with perfusion fluids at body temperature. For maintaining a uniform body and perfusion fluid temperature of 37°C a hydraulic heating pad and a pump are used in the microdialysis procedure (Khan and Shuaib 2001).

Perfusion flow rate

The relative recovery varies inversely with flow rate but this change in recovery is not linear (Alexander et al. 1988, Tossman and Ungerstedt 1986). The recovery falls dramatically at flow rates between 0.2 and 2 μl/min, and then remains relatively constant at flow rates from 2 to 10 μl/min (Alexander et al. 1988). It is recommended that the flow rate should be as low as possible to avoid the rise of the pressure inside the probe and net fluid transport over the membrane. This pressure counteracts the diffusion of molecules into the dialysate (de Lange et al. 1997b). Owing to the mentioned above reasons Benveniste and Hansen (1991) recommend the flow rate of up to 2.5 μl/min. In our experiments with rabbits the flow rate of microdialysis fluid was 1 μl/min (Traczyk et al. 1997, Orlowska-Majdak et al. 2001b, 2003a,b, Kołodziejski et al. 2001)

Dialysis membrane material and surface

The ability of a molecule to pass through the dialysis membrane depends on the chemical nature of the membrane and of the particular molecule. The following types of membrane are used for the construction of the probe: cellulose, regenerated cellulose cuprophan, polycarbonate, polyacetonitril, polyacrilonitril and polysulfone. Of the membranes tested by Maidment and Evans (1991) polyacrylonitrile and the commercial polycarbonate membranes appear most suited for microdialysis of peptides because recovery is the highest for these materials. It is worth mentioning that dialyzed substances may interact with the membrane material. Especially peptides may bind non-specifically or "stick" to various polymers. Out of a number of different probe designs and membranes suitable for microdialysis Kendrick (1991) recommends the commercial probe produced by Carnegie Medicin with polycarbonate membrane. Out of sixteen neuropeptides tested in Carnegie Medicin probe only neurotensin and neuropeptide Y appeared to be "sticky". Moreover, his data *in vitro* showed that the probe performed well for most substances of up to about 3-4 kDa molecular weight. The interaction between polyacrilonitril membrane and serotonin (5HT) (Tao and Hjorth 1992) and polycarbonate membrane and cysteine and glutatione (Landolt et al. 1991) has been described. Permeability of the various probes depends on pore size of the dialysis membrane which varies from 5 to 50 kDa, for the molecular-weight limit of the compounds passing across the dialysis membrane (Levine and Powell 1989). Relative recovery is directly proportional to the size of the dialysis membrane (Neumann et al. 1993, Tossman and Ungerstedt 1986). An over twofold increase in relative recovery *in vitro* of ^{125}I-Oxytocin and ^{125}I-Vasopressin in blood was observed when the microdialysis surface was twofold greater (Neumann et al. 1993).

It is worth mentioning that the choice of dialysis membrane for the probe essential in experiments *in vitro* will generally have little effect on *in vivo* relative recovery. In microdialysis performed *in vitro* the main factor limiting recovery is the membrane resistance to diffusion and the kind of membrane material which is crucial to obtain the best recovery, whereas tissue resistance to diffusion plays a more dominant role in microdialysis performed *in vivo* (Hsiao et al. 1990). Experimental results are consistent with mathematical model of diffusion kinetics of intracerebral microdialysis (Amberg and Lindefors 1989).

Diffusion coefficient

Diffusion of molecules in a solution proceeds completely differently than in tissue. Diffusion in tissue is impeded by cell membranes and thus diffusion pathway is prolonged. Therefore, the recovery depends inversely proportionally on molecular weight of the diffusing substance and inhomogeneity of the tissue and finally, mass transport into the microdialysis probe is less *in vivo* as compared with *in vitro* (Benveniste 1989). Lipophilicity of a diffusing substance and the property of protein binding are also crucial for relative recovery of the substance during microdialysis because they determine the distribution kinetics of the substance in the tissue. An often cited example are the barbiturates where the most lipophylic compounds have a short latency of action due to rapid distribution from blood to the brain. A different distribution of carbamazepine and carbamazepine epoxide in the human brain (Scheyer et al. 1994) and atenolol (hydrophilic) and acetaminophen (moderately lipophylic) in the rat brain (de Lange et al. 1995a) was also shown using intracerebral microdialysis. The authors discussed the results on the basis of different lipophilicity of the mentioned substances.

Composition of the perfusion medium

It is now clear that the composition of the perfusion solution is a critical factor in brain microdialysis. As a fundamental rule the perfusion fluids should be isotonic to blood and should contain the relevant physiological ions consistently with cerebrospinal fluid or brain extracellular electrolyte composition. Microdialysis perfusion medium has a direct access to the extracellular fluid and may locally influence its composition and, finally, the microdialysis data. An increase in NaCl concentration in extracellular fluid caused a release of many amino acids and neurotransmitters, especially dopamine in the rat brain. The authors have shown that it was the response to changed ionic concentration rather than to the osmolality (Horn et al. 1995). Dopamine in striatal dialysates appeared to be very sensitive also to the calcium concentration in the extracellular fluid (Westerink et al. 1988). Moreover, the critical role of this ion in synaptic release is well known. An enhanced concentration of potassium in the medium perfusing the microdialysis probe markedly increased the neuronal electrical activity in the rat brain (Ludvig et al. 1994). A high potassium level is a well known depolarizing agent. When it was applied into the fluid dialyzing caudate nucleus it enhanced the release of methionine--enkephalin in this structure in the sheep (Hashizume et al. 1994), and when applied *via* microdialysis probes into the caudate nucleus and hippocampus in the rabbit, it enhanced vasopressin release (Orlowska-Majdak et al. 2003b). Influence of glucose concentration in the perfusion fluids on dialysate metabolite in the rat brain was studied by Ronne-Engstrom et al. (1995). They concluded that microdialysis with glucose-free perfusion fluid did not change dialysate lactate, pyruvate, aspartate and glutamate concentrations in the rat brain during basal or hypoxic conditions. Maidment and Evans (1991) omitted glucose in the perfusion medium to avoid bacterial growth in the stored microdialysis samples. It is especially important for peptides since bacteria produce peptidases capable of degrading the already low levels of peptides in the samples *in vivo*. Instead of glucose they added bovine serum albumine (BSA) to the dialyzing medium to minimize the sticking of the peptides to the dialysis membrane and tubing, and ascorbic acid as an anti-oxidant (Maidment and Evans 1991). To prevent the breakdown of the peptides in the microdialysis samples bacitracin or aprotinin were added to the perfusion medium (Kendrick 1989). Pich et al. (1993) developed a special procedure to optimize the recovery of corticotropin-releasing factor (CRF) from the mediobasal hypothalamus in the rat. In this procedure the addition of a specific antiserum against CRF to the perfusion medium increased twofold the relative recovery of the CRF *in vitro*.

An artificial cerebrospinal fluid (aCSF) is the medium most frequently used as the perfusion fluid in microdialysis of the brain. However, our laboratory employs 0.9% NaCl instead of aCSF in microdialysis of the rabbit brain (Kołodziejski et al. 2001, Orlowska-Majdak et al. 2001b,

2003a,b, Traczyk et al. 1997) similarly as Lincoln (1992), who employs it for the brain microdialy- sis in ram. We used 0.9% NaCl because of a much earlier loss of efficacy of the probes perfused with aCSF than with 0.9% NaCl solution observed in our first experiments. The cause was probably crystal formation inside the probe from the perfused aCSF during chronic microdialysis. Three solutions were tried as a medium in brain microdialysis in the sheep: 0.9% NaCl, Kreb's Ringer and aCSF and very little difference in the recovery of studied substances was obtained (Kendrick 1991). Simultaneously, Obrenovitch et al. (1995) warned that microdialysis with normal artificial CSF might inhibit propagation of spreading depression in the cerebral cortex by buffering critical changes in the extracellular fluid composition.

ANALYSIS OF DIALYSIS SAMPLES

One of the known disadvantages in the use of the microdialysis technique is the diluting effect of dialysis, which requires sensitive analytical methods to detect small concentrations. The analysis of consecutive dialysate samples by a high-performance liquid chromatography (HPLC), sometimes in combination with enzyme reaction, is the most popular approach. This method uses special detectors for ultraviolet absorbance, mass spectrometry, electrochemistry and fluorescence. HPLC gives the possibility to analyze low molecular-weight neurotransmitters (aminoacids, catecholamines, acetylcholine), metabolites and electrolytes.

To obtain the information about the neurochemical event in the brain as soon as possible, the analysis procedure should be designed at the fastest rate possible. It is especially important in behavioral research, because neurochemical events in the brain can produce behavioral events in time of milliseconds. On-line microdialysis procedure is a fast method for collecting and analyzing the samples (Kissinger 1991). During the whole procedure the samples are not manually handled, but they are automatically passed onto the HPLC column.

Microdialysis coupled to online enzymatic assays has been perfectly reviewed recently (Obrenovitch and Zilkha 2001). These assays include: flow enzyme-fluorescence assays, flow enzyme-amperometric assays and sequential enzyme-amperometric assays. Fluorescence and electrochemical detection have been described but a number of alternative ways of detection can be also considered. The authors suggest replacing the HPLC separation by an online enzyme-based assay for identifying specific compounds in microdialysis samples, especially when high temporal resolution is required. These methods should be suitable for the complementing microdialysis with electrophysiological recording. The analysis of lactate, glucose, ethanol, glutamate, aspartate and D-β-hydroxybutyrate has been described.

Radioimmunoassay (RIA) is in common use for the analysis of peptides. However, there is one great problem with the sensitivity of the RIA. The peptides' concentration in microdialysates is usually very low, in the range of picograms per milliliter, so a high sensitivity of the RIA is needed. The quality of the assay depends on the quality of the anti-peptide antibodies used in the RIA. We used anti-AVP antibodies raised in rabbits in our Department (Orlowska-Majdak et al. 2003b).

ACUTE vs. REPEATED MICRODIALYSIS

Although intracerebral implantation of a microdialysis probe causes brain tissue reaction, it is minimal, restricted to the close vicinity of the probe (de Lange et al. 1995b). Such processes might enhance the blood brain barrier (BBB) permeability at 24 h after implantation of a microdialysis probe because plasma constituents and drugs with little or no passage through an intact BBB increased access to the brain from the surroundings of the dialysis probe (Westergren et al. 1995). Evan's blue, the BBB tracer, which *in vivo* binds to serum albumin crossed the BBB and was observed around the dialysis probe 1, 3 and 7 days but not 21 days after the probe insertion; albumin immunoreactivity was widespread at all times (Johansson et al. 1995). However, Benveniste et al (1984), using α-aminoisobutyrate, demonstrated that the BBB remained intact 30 min after insertion of the microdialysis probe, similarly as Tossman and Ungerstedt (1986) who demonstrated its tightness during 80 min by the sodium technetate procedure. Goldsmith et al. (1995) indicated that 2 h after implantation of the microdialysis probe the BBB was re-established. Although mild neuronal cell death and small petechial hemorrhages were observed in close proximity to the implantation site in the rat hippocampus, the striking finding was the presence of degenerating axons both adjacent to the implantation site and at remote sites (Shuaib et al. 1990). Cellular reaction to the implantation of a microdialysis probe into the rat hippocampus was described also by Benveniste and Diemer (1987). Within the first days they stated only occasional hemor-

rhages surrounding the microdialysis tube and hypertrophic astrocyte processes invading the dialysis membrane, and within two months collagen deposits and occasional granuloma formation in the studied brain tissue. Some brain structures, i.e., the hippocampus but not the striatum, responded by the regional extracellular release of neurotrophic activity to mechanical injury caused by the microdialysis probe implanted into the structure (Humpel et al. 1995). Increased local glucose metabolism and decreased local blood flow found in the close vicinity of the implanted probe normalized after 24 h (Benveniste et al. 1987). Based on these data, the optimal time for commencing microdialysis has been determined to be 24-48 h after the probe implantation (Benveniste and Diemer 1987).

Much more pronounced reactions were found after repeated perfusion procedures (de Lange et al. 1995b). Vacuolization of the white matter, cell death, infiltration of granulocytes, hypercellularity including reactive gliosis, hyaline proteinaceous exudates and hemorrhage indicated an inflammation response of the brain tissue on repeated 4 day dialysis. An increased number of dialysis procedures were paralleled by an increase in hypercellularity and infiltration of granulocytes (de Lange et al. 1995b). This may influence BBB functionality to a large extent. Two opposing data on resting release of amino acids during chronic microdialysis of the brain tissue are frequently cited: a steady increase in the release over a period of 9 days (Korf and Venema 1985) and nearly constant release over a period of 10 months (Delgado et al. 1984). For nonapeptides, the recovery in the microdialysates lowered with the amount of time for which the microdialysis probe remained in the brain and with the amount of perfusion procedures (Kalsbeek and Buijs 1996). Horn and Edelmann's (2001) studies demonstrated that nonapeptides' release into the hypothalamus was reduced by 50-85% if the probes were chronically placed in the brain for 3 or 5 days and perfused. They explained the phenomenon by an increasing diffusion barrier for the nonapeptide molecules. The barrier was determined by the number of glial cells, granulocytes and connective tissue coating the dialysis membrane. However they stated, that for a variety of reasons, they preferred to implant the microdialysis probes chronically (Horn and Engelmann 2001).

Some scientists remove the microdialysis probe at the end of daily microdialysis and reinsert on the next days in chronic experiments to avoid growing of the astrocyte processes into the dialysis membrane and inflammatory reaction that may occur around permanently implanted probes (Páez and Hernández 1996, Reynolds et al. 1999). I do not recommend such proceedings because of multiple irritations of the brain tissue during successive implantations and possibly greater tissue damage than during a single introduction. Such probable damage caused by two-time probe insertions into the striatum was showed by Camp and Robinson (1992). The authors stated that multiple probe insertions might not prove a feasible strategy for dialysis experiments over extended periods of time.

Microdialysis was adapted also to human brain biochemical analysis and drugs' delivery directly into selected tissue (Stahl et al. 2002). Acute microdialysis in the human brain was initially performed by Meyerson et al. (1990) and the first, long-term, 9 days lasting intracerebral microdialysis was performed by Persson and Hillered (1992). Recently, a 16 day intraparenchymal microdialysis was described in a patient with meningocephalitis (Gliemroth et al. 2002).

In our laboratory, we used chronic microdialysis of the brain structures in awake rabbits during five years' studies on the role of neuropeptides in memory processes. The probes (CMA/Microdialysis) remained in the brain and the microdialysis was regularly repeated, on the average, for up to two - three months (Orlowska-Majdak et al. 2003b). Histological examination of each dialyzed brain at the end of the experimental protocol showed evident hypercellularity around the track left by the microdialysis probe. However, Ianus Green solution perfused through microdialysis probes just before the animals' death crossed the dialysis membrane and showed the great spatial distribution in the neighboring brain tissue. Apparently the great concentration of cells shown round the site of probe implantation did not disturb the molecules of dye to diffuse deeply into the brain tissue. Nor did it disturb diffusing molecules of endogenous brain AVP into the fluid perfusing microdialysis probe implanted into the hippocampus and caudate nucleus, because we could determine it in the outflowing fluid all the time of repeated microdialysis procedure (Orlowska-Majdak et al. 2003b). Moreover, we observed the effect of AVP (Orlowska-Majdak et al. 2001b) and TRH (Kołodziejski et al. 2001) dialyzed into the rabbit hippocampus on the memory processes for up to two months since the probe implantation; it means that molecules of the mentioned neuropeptides crossed the microdialysis membrane and diffused into the brain structure during all the time of microdialysis experiment.

An essential problem in chronic microdialysis experiments is keeping the probe's patency. In our experiments CMA/ Microdialysis probes perfused with 0.9% NaCl solution remained in the rabbit brain and preserved their efficacy for up to two – three months. The longest time of the probe functioning amounted to five months (Orlowska-Majdak et al. 2003b). Lincoln (1992) described microdialysis experiments where the probes worked for up to 10 weeks. The longest time of chronic microdialyis, up to 10 months, using a dialytrode, was described by Delgado et al. (1984).

MICRODIALYSIS vs. OTHER *IN VIVO* RELEASE MODELS

Neurotransmitters become biologically active only after their release into the extracellular space. There are two groups of methods available for the *in vivo* intracranial analysis of the released substances, that is voltammetry and perfusion methods. These techniques are superior to the detection of substance concentrations in tissue homogenates that do not provide information about whether the analyzed neurotransmitter is located intra- or extracellularly.

Voltammetry measures the oxidation current from electroactive molecules in the extracellular fluid. In this approach a three electrode system (consisting of a working, a reference and an auxiliary electrode) is implanted in a discrete area of the brain (de Lange et al. 1997b). Unfortunately, only a limited number of drugs can be oxidized at a reasonable oxidation potential, and this limits the applicability of this technique. Catecholamines are relatively easy to oxidize. Voltammetry has a temporal resolution of the order of seconds, so it is a very fast method. It can be used to study stimulated release of substances, especially electrically stimulated and permits the observation of rapidly changing concentrations. A variety of voltammetric techniques have been used *in vivo*, including chronoamperometry, linear potential sweep voltammetry, differential pulse voltammetry (DPV) and differential normal pulse voltammetry (DNPV) (Khan and Shuaib 2001).

In the awake animals perfusion is applied as the push-pull perfusion or microdialysis. Both methods allow the study of neuropeptides release into the brain structures in the awake animals under various conditions. They have a much longer sampling period than voltammetry, so they are better in behavioral research. Local changes should primarily reflect changes in the release patterns from basal levels to that associated with behavior and pharmacological treatments so it can be well correlated to changes in physiological processes and behavioral performance. Moreover, microdialysis can be considered to be a preferable technique in behavioral research simply because it is easier; it allows to avoid problems with great tissue damage associated with the push-pull perfusion. Microdialysis preserves the anatomical and functional integrity of the surrounding tissue better than does the push-pull perfusion. The chronically implanted dialysis probes make it convenient to carry out repeated microdialysis for days and weeks. Push-pull perfusions are usually carried out in acute experiments. Moreover, dialysis membrane acts as a filter against the large molecules present in the extracellular fluid that could disturb in further analysis of the samples. Furthermore, the dialysis probes allow the use of inverse microdialysis (retrodialysis) approaches to simultaneously administer exogenous substances, including antagonists, without acutely disturbing the animal's behavior.

APPLICATION OF BRAIN MICRODIALYSIS IN THE STUDY OF VASOPRESSIN AND OXYTOCIN

Both push-pull perfusion and microdialysis are used in AVP and OXT research but only three scientific centers i.e., German, Polish and Japanese, applied microdialysis in the studies on behavior-modulating action of AVP and OXT.

Active avoidance

Engelmann et al. (1992) applied AVP and V_1 or V_2/V_1 antagonist into the septum via microdialysis and examined the acquisition of pole jumping behavior in rats. They discovered the participation of AVP and V_1 receptors in this behavior.

Passive avoidance

Synthetic AVP or V_1/V_2 receptor antagonist were microdialysed into the mediolateral septum of pinealectomized and sham operated rats and the latency of passive avoidance response was measured. Intraseptal AVP administration facilitated the avoidance response only in pinealectomized rats which means that septal AVP modulates passive avoidance be-

havior *via* the pineal gland and probably through melatonin (Appenrodt and Schwarzberg 1999).

Social recognition

Engelmann et al. (1994) reported a correlation of intra-SON and intra-septal release of AVP and social recognition improvement in rats in another microdialysis study. The effect was V_1 receptor-dependent. Then the role of septum in the effect of AVP on social recognition and participation of V_1 receptors was studied in Long-Evans and Brattleboro rats (Engelmann and Landgraf 1994). Microdialysis administration of synthetic AVP into the septum significantly improved social recognition in both rat strains, and V_1 antagonist impaired social recognition in Long-Evans rats (Engelmann and Landgraf 1994). OXT also affected social recognition acting in the olfactory bulb in rats (Dluzen et al. 1998). Microdialysis of the olfactory bulb allowed to reveal the involvement of norepinephrine system and α-adrenoceptors in such effect of OXT (Dluzen et al. 2000).

Spatial memory

Synthetic AVP or V_1 receptor antagonist were microdialysed into the septum in the rat and their influence on spatial memory was tested in the Morris water maze procedure. The results of this experiment were incoherent, because application of V_1 antagonist was ineffective whereas synthetic AVP impaired place navigation learning (Engelmann et al. 1992). Japanese scientists studied the mechanism by which AVP(4-9) improved learning in the eight-arm radial maze. Rat ventral hippocampus was microdialysed and the concentration of acetylcholine (ACh) was determined in rats with scopolamine-induced impairment of spatial memory. The obtained results suggest that AVP(4-9) microinjected into the ventral hippocampus potentiates ACh release from the structure (Fujiwara et al. 1997) by activation of V_{1A} receptors on the postsynaptic membrane of cholinergic neurons (Mishima et al. 2001).

Anxiety-related behavior

The elevated plus maze test allows a reliable measurement of anxiety in rats. AVP administered into the septum of male rats by means of microdialysis induced anxiolytic-like effect in Liebsch et al. (1996) and Appenrodt et al. (1998) studies. Application of V_1 receptor antagonist instead of AVP brought different results in both studies. A further microdialysis study in pinealectomized rats revealed that although pineal gland and melatonin were not basically involved in the anxiety-related behavior, an intact pineal function seemed to be necessary for anxiolytic effect of AVP (Appenrodt and Schwarzberg 2000).

Eyelid conditioned reflex

Classical eyeblink conditioning, considered as a procedural task is a form of associative learning. We used for the first time this paradigm to study AVP and OXT effect on learning in rabbits. A new experimental set and procedure for rabbit's eyelid conditioning was constructed and described (Orlowska-Majdak et al. 2001a). The peptides were applied *via* microdialysis probes chronically implanted into the hippocampus and caudate nucleus as the reference structure and the percentage of conditioned responses were calculated. Restraining of the process of extinction was shown during AVP dialysis through the hippocampus and caudate nucleus, but the effect in hippocampus was stronger and longer lasting than in the caudate nucleus (Orlowska-Majdak et al. 2001b). OXT applied into the same structures had no effect on eyelid conditioning (Orlowska-Majdak et al. 2003a).

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