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Appraisal of state-of-the-art

Applicability of reverse microdialysis in pharmacological and toxicological studies

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Abstract

A recent application of microdialysis is the introduction of a substance into the extracellular space via the microdialysis probe. The inclusion of a higher amount of a drug in the perfusate allows the drug to diffuse through the microdialysis membrane to the tissue. This technique, actually called as reverse microdialysis, not only allows the local administration of a substance but also permits the simultaneous sampling of the extracellular levels of endogenous compounds. Local effects of exogenous compounds have been studied in the central nervous system, hepatic tissue, dermis, heart and corpora luteae of experimental animals by means of reverse microdialysis. In central nervous studies, reverse microdialysis has been extensively used for the study of the effects on neurotransmission at different central nuclei of diverse pharmacological and toxicological agents, such as antidepressants, antipsychotics, antiparkinsonians, hallucinogens, drugs of abuse and experimental drugs. In the clinical setting, reverse microdialysis has been used for the study of local effects of drugs in the adipose tissue, skeletal muscle and dermis. The aim of this review is to describe the principles of the reverse microdialysis, to compare the technique with other available methods and finally to describe the applicability of reverse microdialysis in the study of drugs properties both in basic and clinical research.
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Keywords: Reverse microdialysis; Delivery methods; Sampling methods; Pharmacological and toxicological agents; Basic research; Clinical applications; Neurotransmission

1. Introduction

Microdialysis sampling is an increasingly employed research method for the study of pharmacokinetic and pharmacodynamic behavior of therapeutic and toxicological agents (Höcht, Opezzo, & Taira, 2004). Traditionally, microdialysis has been used for the determination of interstitial levels of endogenous compounds and drugs. Actually, microdialysis has found important application in the field of blood pharmacokinetics, tissue distribution of drugs, pharmacokinetic–pharmacodynamic (PK–PD) modelling so much in animals as in human (Höcht et al., 2004).

A more recent application of microdialysis is the introduction of a substance into the extracellular space via the microdialysis probe (Galvan, Smith, & Wichmann, 2003). The inclusion of a higher amount of a drug in the perfusate allows the drug to diffuse through the microdialysis membrane into the tissue. This technique is actually called as reverse microdialysis (Chan & Chan, 1999) (Fig. 1).

Reverse microdialysis, based on the dialysis principle, not only allows the local administration of a substance but also permits the simultaneous sampling of the extracellular levels of endogenous compounds (Fig. 1). Therefore, reverse microdialysis serves both as an administration and sampling technique. To date, reverse microdialysis has been mainly used for the study of the effect of local drug administration in brain concentration of neurotransmitters and metabolites. However, the effect of drugs in other tissues, such as liver, dermis, corpora lutea and heart has also been studied by means of reverse microdialysis. More recently, reverse microdialysis has been introduced for the evaluation of pharmacodynamics of therapeutic agents in the clinical setting. The large body of work published (more than 300 publications) underscores the importance of the reverse microdialysis technique for the study of pharmacological and toxicological aspects of therapeutic drugs.

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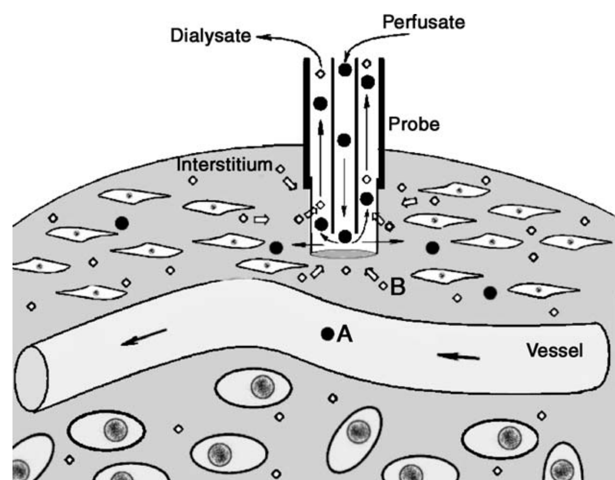

Fig. 1. Representation of the reverse microdialysis principle. A concentric microdialysis probe inserted in the extracellular space of a tissue is perfused with a solution containing the exogenous compound (black circles) to administer. The solution is pumped through the inner cannula allowing the diffusion of the exogenous compounds to the surrounding tissue. Simultaneously, endogenous compounds (white rhombus) diffuse from the extracellular space into the microdialysis probe and are collected in the dialysate for further analysis.

The aim of this review is to describe the principles of the reverse microdialysis, to compare the technique with other available methods and finally to describe the applicability of reverse microdialysis in the study of drugs properties in both basic and clinical research.

2. Principles of reverse microdialysis

In the past twenty years the microdialysis technique has become a method of choice in the study of tissue concentrations of both endogenous and exogenous substances. The microdialysis sampling technique, as we know it today emerged from the neurosciences, where it was originally used for measuring concentrations of neurotransmitters in rat brain (Ungerstedt & Pycock, 1974).

In this technique, a probe that is inserted into tissue mimics the function of a capillary blood vessel (Fig. 1). The probe has a hollow fiber that is permeable to water and small molecules, and when it is perfused with a physiologic fluid, molecules are exchanged through the dialysis membrane by diffusion in both directions in favour of a gradient of concentration. Later, dialysate samples are analyzed using highly sensitive techniques. Therefore, microdialysis samples endogenous compounds, because levels of neurotransmitters and metabolites are higher in the extracellular space than in the perfusion fluid. On the other hand, the inclusion of a higher concentration of a drug or endogenous compound in the perfusate allows the substance to diffuse through the dialysis membrane to the tissue (Höcht et al., 2004).

The microdialysis technique is not performed under equilibrium conditions because the perfusate is constantly being pumped through the probe, and therefore the concentration of the endogenous compounds in the dialysate is some fraction of that in the surrounding tissue. This fraction is called relative recovery (Plock & Kloft, 2005). Also, the concentration of the drug administered through the microdialysis probe is a fraction of the concentration of the same in the perfusate.

The basic setup for a microdialysis experiment consists of a microdialysis probe, a perfusion pump, and an analytical method with the required sensitivity to quantify small concentrations of substances in small volumes of sample (de Lange, de Boer, & Breimer, 2000).

3. Methodological considerations

3.1. Microdialysis probe

The microdialysis probe is perhaps the nucleus of the microdialysis experiment. The microdialysis probe typically consists of a tubular dialysis fiber that is connected with an inlet and an outlet tube. The inlet and outlet tubes are connected with thin and flexible tubing with a perfusion pump and with a fraction collector, respectively. It is very important that they do not interact with the perfusate or the surrounding tissue. Many laboratories have designed their own probe as its construction lasts not longer than several minutes but today there are commercial approved probes for studies in human soft tissues and brain.

In general, probes have a longitudinal, a semicircular or an I-shape design. Various designs have been described: concentric cannula probe, linear probe, shunt probe. Several modified probes designs such as: spinal loop dialysis catheter (Marsala, Malmberg, & Yaksh, 1995), flexible intravenous probe (Evrard, Cumps, & Verbeeck, 1996) shunt intraarterial microdialysis probe (Höcht, Opezzo, & Taira, 2003) has also been reported. Microdialysis probes have been described in previous reviews (Höcht et al., 2004; Plock & Kloft, 2005) and are beyond the scope of the manuscript.

3.2. Microdialysis membrane

The choice of the membrane type and size is an essential element to optimize the microdialysis probe for a particular experiment. Conventional microdialysis probes are constructed with 20 kDa molecular weight cut-off membranes enabling the measurement of small molecules such as glucose, lactate, pyruvate and glutamate. Common substances used as membrane materials are cuproamonic rayon, celluloses, polycarbonate, polyethersulfone or cuprophan (Höcht et al., 2004). Recently, a 100 kDa molecular weight cut-off microdialysis membrane has been introduced to allow detection of larger molecules such as cytokines (Hutchinson et al., 2005).

Important aspect to be considered in a reverse microdialysis experiments with regard to the dialysis membrane is the molecular weight of the compounds to be administered or sampled, the size of the microdialysis membrane and the interaction of the membrane with the perfusate or the surrounding tissue.

Despite the molecular weight cut-off of the membrane, the molar mass of the substance of interest has to be taken into consideration. As discussed previously, only substances with a

molar mass lower than the weight cut-off are capable of passing the membrane. However, even if the molar mass falls below the molecular weight cut-off, an acceptable relative recovery will only be attained with substances having a molar mass lower than approximately one-fourth of the membrane cut-off (Plock & Kloft, 2005).

The size of the microdialysis membrane can also influence the relative recovery. According to Fick's law of diffusion the rate of perfusion across a membrane is proportional to its area. Therefore, increasing the length and thus the area of the microdialysis membrane will lead to an increase in relative recovery (Plock & Kloft, 2005). On the other hand, it has been reported that increasing the outer diameter of the inner cannula may enhance relative recovery of the probe (Torto, Mikeladze, Gorton, Csoregi, & Laurell, 1999; Wisniewski & Torto, 2002).

Another important aspect of the membrane size is to limit the drug administration through the probe to a specific tissue. This is especially important in the study of drug effects at the central nervous system. The microdialysis membrane must not exceed the size of the central nuclei to be studied. Therefore, reverse microdialysis has been used for the study of drug effects in relative large central nuclei. The investigator could select the adequate size of the dialysis membrane taking into account the size of the central tissue and that most of the compounds have slow infusion rates and did not diffuse more than 1 mm from the membrane into the tissue (Westerink & de Vries, 2001).

Recently, Tsou et al. (1994) have reported the construction of a microdialysis probe with an active exchange area of the membrane of 200 mm in length and 220 mm of diameter. The membrane is made of regenerated cellulose and has a cut-off of 6000 Da. The microdialysis probe exhibits a high in vitro recovery, making it suitable for the study of small central nuclei.

Another important key is that the membrane does not interact in any way with the surrounding tissue or with the perfusate. Lower recoveries of acid aminoacids due to the presence of surface charge have been described (Sandberg & Lindström, 1983). Also recovery of neuropeptides can vary as much as 20% with different dialysis membranes (Kendrick, 1989).

3.3. Perfusion fluids

Perfusion media used in microdialysis experiments vary widely in composition and pH (Table 1). The ideally composition, ion strength, osmotic value and pH of the perfusion solution should be as close as possible to those of the extracellular fluid of the dialyzed tissue. The perfusate is an aqueous solution of sodium and potassium salts and other ions in a minor proportion, without proteins or a very small concentration of it. In some cases, proteins should be added to the perfusion medium to prevent sticking of drugs to the microdialysis probe and tubing connections (Maidment, Brumbaugh, Rudolph, Erdelyi, & Evans, 1989).

It is important to ensure an accurate concentration of calcium in the perfusion solution. An increase of the calcium concentration of 65% in the perfusate with regards to the interstitial space increases dopamine dialysate concentrations by 70% (Moghaddam & Bunney, 1989). Most of the investigators that used microdialysis for neurotransmitters sampling employed perfusion solutions with a high calcium concentration to ensure endogenous compounds quantification.

Table 1
Different compositions of perfusion solutions (Höcht et al., 2004)

Perfusion medium	Composition
Destilled water	
Saline	0.9% NaCl
	0.9% NaCl; 0.5% bovine serum albumin
Ringer solution	147 mM NaCl; 1.3 mM $CaCl_2$; 4 mM KCl (pH 7.2)
Modified Ringers solution	145 mM NaCl; 1.2 mM $CaCl_2$; 2.7 mM KCl, 1 mM $MgCl_2$; 0.2 mM ascorbate (pH 7.4)
Buffered Ringers solution	147 mM NaCl; 3.4 mM $CaCl_2$; 2.8 mM KCl, 1.2 mM $MgCl_2$; 0.6 mM K_2HPO_4; 114 mM ascorbate (pH 6.9)
Krebs Ringer solution	138 mM NaCl; 1 mM $CaCl_2$; 5 mM KCl; 1 mM $MgCl_2$; 11 mM $NaHCO_3$; 1 mM Na_2HPO_4, 11 mM glucose (pH 7.5)
Krebs Ringer bicarbonate	122 mM NaCl; 1.2 mM $CaCl_2$; 3 mM KCl, 1.2 mM $MgSO_4$; 25 mM $NaHCO_3$; 0.4 mM KH_2PO_4, (pH 7.4)
Krebs–Henseleit bicarbonate buffer	118 mM NaCl; 2.5 mM $CaCl_2$; 4.7 mM KCl, 0.6 mM $MgSO_4$; 25 mM $NaHCO_3$; 1.2 mM NaH_2PO_4, 11 mM glucose
Mock-cerebrospinal fluids	127 mM NaCl; 1.1 mM $CaCl_2$; 2.4 mM KCl; 0.85 mM $MgCl_2$; 28 mM $NaHCO_3$; 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4; 5.9 mM glucose (pH 7.5)
Bile salt Ringer's	155 mM NaCl; 5.5 mM KCl; 2.3 mM $CaCl_2$; 20 mg ml^{-1} bile salts.

Also, most investigators use a perfusion medium at room temperature before entering the probe. As a result a temperature gradient exists between the probe and its environment. This may have an effect on tissue processes and consequently on the results.

Another important issue in reverse microdialysis experiment is to ensure that the added substance did not change pH or tonicity of the perfusion medium, because these alterations could affect transmitters release and consequently the results of the study (Ungerstedt, 1991). However, a small concentration of the compound added to the perfusion solution is unlikely to change the perfusion fluid properties.

3.4. Analytical aspects

An important aspect of reverse microdialysis studies is the selection of an adequate analytical method for endogenous compound determination. Microdialysis generates small volume samples (1–10 µl), because of the need of slow perfusion rates (0.1–2 µl) to obtain high recoveries of the endogenous compounds maintaining an adequate temporal resolution. Also, endogenous compounds, especially neurotransmitters, are often contained at very low concentration in the interstitial space (pM–fM range). Thus, the limit of detection of the analytical method must be less than the lowest expected in vivo concentration.

A wide range of analytical methods can be used for the analysis of the microdialysis samples (Davies, Cooper, Desmond, Lunte, & Lunte, 2000). Non-separation-based methods allow the detection of one analyte at a time, in contrast with separation-based methods that can be used for the detection of multiple analyte (neurotransmitters and its metabolites) in each sample. Therefore, the last method is the most adequate for reverse microdialysis studies.

The separation-based methods available for analysis of microdialysis samples are: liquid chromatography (LC), microbore LC, capillary LC and capillary electrophoresis (CE). There are a wide range of different detectors coupled to the separation method. Immunoassay, ultraviolet absorbance (UV), electrochemical, fluorescence and mass spectrometric are the most common detectors for LC analysis (Davies et al., 2000). For CE, electrochemical and laser-induced fluorescence (LIF) detection have been most commonly employed.

The drawback of the separation-based methods is the dilution of the microdialysis sample. In comparison to the non-separation-based methods, this method allows the detection of many compounds in each sample. Therefore, the investigator can monitor not only the extracellular levels of the drug, but also the concentration of endogenous compounds.

For improve the sensitivity of the LC method, it is not only important to select the adequate detector but also the type of column. Microbore columns have become popular for the analysis of microdialysis samples, because these columns result in an increase of sensitivity due to the smaller length and inner diameter (Pettit & Justice, 1991). This means that the microdialysis samples undergo less dilution with microbore columns with regards to conventional columns. With the use of microbore columns, there is a need to reduce at minimum dead volumes in the injector, microbore column and detector connections to preserve peak resolution (Cheng & Kuo, 1995). Requirement of low flow rates (usually 0.1 ml min^{-1}) for microbore chromatography is an important aspect. To resolve this issue there are commercially available pumps with low flow rates as well as flow splitting systems.

Capillary LC has been also employed for the analysis of microdialysis samples. One advantage of capillary LC columns is that relatively large volumes of analytes can be injected on the column and then eluted in a very small volume using gradient elution (Boyd, Witowski, & Kennedy, 2000).

Despite the advantage of microbore and capillary LC, conventional columns are still more commonly employed because they do not need special instrumentation such flow rate pumps, low dead volume injectors or reduced volume detection cells. However, the investigator should know the availability of these special systems if greater sensibility of the analytical method is needed.

CE is becoming increasingly popular as an analysis method for microdialysis samples. Although capillary electrophoresis only need 1–10 nl of injection volumes for off-line analysis, 1–5 μl samples are generally required due to difficulties with physical manipulation. One disadvantage of CE is that the high ionic strength of microdialysis samples reduces detection sensitivity (Davies et al., 2000).

3.5. Quantification aspects

An accurate calibration of the microdialysis probe is necessary for pharmacokinetic experiments using microdialysis sampling, because the desired information is the absolute drug concentration in different tissues. The relationship between the concentration of the drug in the dialysate and the concentration of the drug in the sample matrix may be thought of as the recovery of the probe.

Recovery of the microdialysis probe can be determined with in vitro and in vivo assays. The in vitro estimation of the probe recovery, serves only to prove if the microdialysis probe works, because in vitro recovery values are often an overestimation of the in vivo recovery. This is explained because the nature of the tissue to be sampled and its interactions with the drug diminished the recovery (Benveniste, Hansen, & Ottosen, 1989; Ståhle, Segersvärd, & Ungerstedt, 1991). In most cases the diffusion of the drug through the tissue is the rate limiting step in the recovery of the drug. So, the determination of in vivo recovery of microdialysis probe is essential for the accurate estimation of drug concentration in the different tissues.

Contrary to the pharmacokinetics studies, accurate calibration of the microdialysis probe in pharmacodynamic studies is not necessary, because the desirable information is the relative change of the concentration induced by drug administration. So, only the concentration independence and stability of the recovery need to be known. These properties could be determined by in vitro calibration of the microdialysis probe. On the other hand, in vivo recovery of the microdialysis probes is constant during the microdialysis experiment (Edwards, Brouwer, & McNamara, 2002).

This way, an initial period for the determination of basal dialysate concentration of the endogenous compound is needed. After implantation of the microdialysis probe, there is an initial period of disturbed tissue function due to lesion of the surrounding tissue altering extracellular levels of endogenous compounds. In our experience in central nervous system studies, the compound levels are altered during the first hour after probe implantation. After this period, the basal concentration of the endogenous substance in approximately four microdialysis samples must be determined. After basal levels sampling, the drug could be administered and the change in the endogenous substance induced by the drug could be calculated as a percentage of the basal mean.

If the neurotransmitters are not able to be detected by the analytical technique, it could be used the change of metabolites of neurotransmitters as a tool for studies of drugs with action on neurotransmission (Church & Justice, 1987; Sharp, Zetterstrom, & Ungerstedt, 1986; Zetterstrom, Sharp, & Ungerstedt, 1986). Levels of metabolites in microdialysis samples are several times higher than neurotransmitters concentrations. So, studies had shown that the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) in the dialysate reflect the magnitude of the intraneuronal catabolism of the dopamine after their uptake (Alexander et al., 1988; Sharp et al., 1986; Zetterstrom et al., 1986). Consequently, the determination of dopaminergic metabolites in dialysate samples provides an index of the in

vivo dopaminergic metabolism and turnover. However, metabolites are slower indicators of changes in neuronal activity and represent more long-term changes in the release of neurotransmitters (Bourne, 2003).

3.6. Determination of the amount of drug administered by reverse microdialysis

One advantage of the reverse microdialysis with respect to other methods for drug delivery is the possibility to manipulate and estimate the amount of drug that diffuse into the extracellular space.

Amount of the added substance that is delivered to the interstitial space could be calculated by the determination of the concentration of the compound in the perfusate and the dialysate. The difference of these concentrations represents the amount of drug delivered into the tissue during the sampling time. Therefore, the sum of the difference of concentrations between perfusate and dialysate of the drug for each sampling interval represent the total amount of drug delivered during the reverse microdialysis experiments (Chan & Chan, 1999).

The total amount of the applied drug can be determined with the following equation:

$$D_{\mathrm{A}} = \sum_{i=1}^{n} (C_{\mathrm{per}} - C_{\mathrm{dial}})^{*} R^{*} \Delta t$$

where n is the succession of sampling, i.1 to n, D_{A} is the total amount of the drug A administered via reverse microdialysis, C_{per} is the concentration of A in the perfusate, C_{dial} is the concentration of A in the dialysate, R is the flow rate of perfusion and Δt is the time of the sampling interval.

In the reverse microdialysis technique, drug administration into the site of perfusion is of zero order similar to an intravenous constant rate infusion. So, constant levels at the site of perfusion are attained when the diffusion rate of the drug from the tissue surrounding the microdialysis probe due to distribution in other tissues is similar to the diffusion rate of the drug from the probe to the extracellular space.

3.7. Tissue damage by microdialysis probe implantation

Tissue disturbance originated by microdialysis probe implantation initially induced a release of neurotransmitters in the surrounding area of probe implantation. However, neurotransmitters rapidly returned to basal levels. There is also an initial period of disturbed tissue function that lasts from 30 min to 24 h, characterized by increased glucose metabolism, decreased blood flow and disturbed neurotransmitters release (Ungerstedt, 1991). On the other hand, microdialysis probe implantation has only minor effects on blood brain barrier integrity (Benveniste & Hansen, 1991).

After chronically implantation of a microdialysis probe for 2 days, histological studies have shown the presence of oedema, minor haemorrhages and leukocytes accumulation in the surrounding tissue of the probe (Benveniste & Hansen, 1991).

Therefore, the investigator must consider these acute and chronic tissue alterations in the design of a reverse microdialysis study.

3.8. Anaesthetized versus awake animal model

A reverse microdialysis experiment can be realized in anaesthetized or freely moving animals. With the use of anaesthetic agents, it must be evaluated the effect of the anaesthetic preparation on the pharmacological response of the applied drug and its effect on specific transmitters systems (Claassen, 1994). In general, anaesthetized animals are more suitable for experiments with more than one microdialysis probe, local injection cannulas and in experiments requiring dissections (Ungerstedt, 1991).

In reverse microdialysis experiments conducted in awake animals, the investigator should consider that stress could introduce artefacts in the measurements. So, environmental stress needs to be evaluated (Bourne, 2003).

4. Comparison of reverse microdialysis with other drug delivery techniques

Reverse microdialysis is an interesting alternative to other methods of drug delivery, such as microinfusion through an implanted intracranial cannula. Drug administration by microinfusion has several disadvantages comparing with reverse microdialysis. In microinfusion studies the compound is administered by the introduction of a determined volume of fluid into the extracellular space of the tissue of interest. As volume expansion occurs, this technique is rather useful for chronic drug administration, especially for chronic infusion into brain parenchyma (Bazzett, Becker, & Albin, 1991). Therefore, microinfusion may produce non-specific tissue damage due to the volume of fluid introduced. On the contrary, reverse microdialysis delivered the drug without net gain of fluid making this technique suitable for chronic drug administration. It is only important to ensure that the recovery of the microdialysis probe did not change during the experiment because of alterations of the surrounding tissue such as gliosis or inflammation.

Moreover, microinfusion provides only a point source of drug targeting a limited area of the tissue of interest. On the other hand, once a drug has been given, the same diffused rapidly out of the brain into the peripheral bloodstream making interpretation of results extremely difficult (Evans, Armstrong, Singer, Cook, & Burnstock, 1975). With the reverse microdialysis technique, the drug is chronically perfused into the tissue maintaining more constant drug concentration at the target tissue. In addition, the drug targeted a larger volume of the tissue because the drug diffused to the interstitial space from the whole microdialysis membrane. Therefore, an important aspect in reverse microdialysis experiment is the selection of the adequate membrane size in order to ensure drug administration a great volume of the target tissue.

Solutions injected by microinfusion through an intracranial implanted cannula are assumed to enter the brain as a spherical

drop, making that variation in drop size, resulting from pressure injection delivery, may produce variability in the diffusion area (Rice, Gerhardt, Hierl, Nagy, & Adams, 1985).

Another advantage of reverse microdialysis drug delivery is the potential to monitor the proper operation of the microdialysis probe throughout a period of chronic drug administration. In intracranial injection studies, incomplete expulsion due to cannula obstruction has been reported (Hargraves & Freed, 1987). On the other hand, reverse microdialysis is more suitable for dose–response studies in the same animal, because there is no need to reimplant the microdialysis probe for further dose administrations.

The most important drawback of reverse microdialysis with regards to other delivery methods is the greater cost of the equipment.

5. Comparison of reverse microdialysis with other sampling techniques

Push–pull perfusion and in vivo voltammetry are alternative sampling methods to reverse microdialysis. However, these techniques did not allow drug administration.

Push–pull probes consist of two concentric tubes so that the perfusion fluid is introduced by means of a pump through the inner tube and removed by a second pump through the outer tube (Westerink & Justice, 1991). One advantage of this method with regards to microdialysis is that the recovery of endogenous compounds is greater and allows a higher output of the recorded endogenous substance. The absence of a membrane barrier in push–pull probes makes this method more adequate for the recovery of compounds which adsorb to a dialysis membrane or which hardly diffuse through the membrane such as peptides (Westerink & Justice, 1991).

However, push–pull perfusion has several drawbacks with regards to microdialysis sampling. In push–pull studies, high flow rates are required to prevent blockage of fluid flow generating turbulence and consequently significant tissue damage. On the other hand, push–pull perfusion did not prevent enzymatic degradation of the endogenous compounds and needed clean-up procedures for further analysis. Moreover, push–pull perfusion is technically more complicated than microdialysis perfusion, because two exactly calibrated pumps are necessary to transport both the push and the pull fluids (Westerink & Justice, 1991).

In vivo voltammetry consists of the measurement of electro active compounds at the surface of an implanted carbon electrode (Fillenz, 2005). This technique involves the oxidation or the reduction of an analyte as the result of an applied potential. Although each compound has a characteristic oxidation potential, the differences between oxidation potentials are so small that specificity is a serious challenge. In voltammetry using enzyme-based biosensors, the specificity of the enzyme established the identity of the endogenous compounds. Another limitation of in vivo voltammetry is the fact that only electro active compounds or enzyme specific substance can be determined with this technique. However, comparing with microdialysis sampling, in vivo voltammetry has a higher time resolution and is therefore the most adequate sampling method for behavioral studies (Fillenz, 2005).

6. Applications of reverse microdialysis in pharmacological and toxicological studies

Reverse microdialysis has been extensively employed for the study of local effects of pharmacological and toxicological agents in basic research, especially at the central nervous system (Table 2). In the clinical setting, the effect of lipolytic drugs, adverse drug reactions at the dermis and metabolism of the skeletal muscle has been studied by means of local application of therapeutic agents through a microdialysis catheter (Table 3). In this section, the most recently applications of reverse microdialysis in pharmacological and toxicological studies are discussed.

6.1. Basic research

Local effects of exogenous compounds have been studied in the central nervous system, hepatic tissue, dermis, heart and corpora lutea by means of reverse microdialysis.

Although the most employed species for reverse microdialysis studies is the rat, experiments are also made in hamster, mice, monkey, dog, cow, gerbils and chick (Table 2).

6.1.1. Central nervous system

In central nervous system studies, reverse microdialysis has been extensively used for the study of the effects on neurotransmission at different central nuclei of diverse pharmacological and toxicological agents, such as antidepressants, antipsychotics, antiparkinsonians, hallucinogens, drugs of abuse and experimental drugs (Table 2). The most relevant findings of central reverse microdialysis studies are discussed in the next paragraphs.

See and Berglind (2001) studied the effect of antipsychotics on gamma-aminobutiric acid (GABA) release in the globus pallidus of rats. Whilst, reverse dialysis of clozapine induced a concentration dependent decrease in extracellular GABA, haloperidol perfusion did not affect extracellular levels of the neurotransmitter. The authors concluded that clozapine has direct actions within the globus pallidus, while the effects of haloperidol are most likely mediated through its action in the striatum. The ability of clozapine to effectively decrease pallidal GABA release may constitute the mechanism by which this antipsychotic lacks the motor side effects that commonly characterize neuroleptics such as haloperidol. Therefore, reverse microdialysis is a powerful tool for the study of differential pharmacological actions at the central nervous system of antipsychotic drugs.

The effect of morphine in periaqueductal gray matter was studied by perfusing the nuclei with the drug and simultaneously recovering GABA in the dialysate (Stiller, Bergquist, Beck, Ekman, & Brodin, 1996). The authors found that morphine perfusion significantly decreased GABA dialysate levels and this effect was reversed by

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Table 2
Overview of recently published studies using the reverse microdialysis technique in basic research

Drug	Tissue	Species	Analytical determination	Effects on other parameters	Reference
8-OH-DPAT	Nucleus accumbens	Rat	5-HT, 5-HIAA, DOPAC		Müller et al., 2004.
ACPD	Prefrontal cortex	Rat	GABA, ACh		Segovia & Mora, 2005.
Amytriptiline	Hindpaw	Rat	Adenosine		Sawynok et al., 2005.
Atipamezole, medetomidine	Paraventricular nucleus and arcuate nucleus of hypothalamus	Siberian hamster	Prolactin		Dodge & Badura, 2002.
Capsaicin	Dorsal horn	Rat	Substance-P		Warsame Afrah et al., 2004.
Citalopram	Dorsal raphe nucleus	Rat	5-HT		Tao, Ma, & Auerbach, 2000.
Citalopram, GBR-12909	Dorsal hippocampus	Rat	DA, 5-HT	Assessment of seizure severity	Clinckers, Smolders, Meurs, Ebinger, & Michotte, 2004.
Clozapine, haloperidol	Globus pallidus	Rat	GABA		See & Berglind, 2001.
CNQX, SCH 23390, sulpiride, bicuculline	Prefrontal cortex	Rat		Intracellular electrophysiological recordings	Lavin et al., 2005.
DOI	Somatosensory cortex	Rat	Glu		Scruggs, Schmidt, & Deutch, 2003.
Entacapone, tolcapone	Striatum	Rat	DOPAC, HVA		Forsberg, Huotari, Savolainen, & Männistö, 2005.
Ethosuximide	Thalamus	Rat		Electroencephalogram	Richards et al., 2003.
Eticlopride, SCH 23390	Striatum	Rat		Intracellular electrophysiological recordings	West & Grace, 2002.
Fluoxetine, 8-OH-DPAT	Preoptic area and anterior hypothalamus	Rat	5-HT, 5-HIAA	Body temperature	Ishiwata et al., 2004.
Iptakalim	Striatum	Rat	DA, Glu, DOPAC		Yang et al., 2006.
Irbesartan	Anterior hypothalamus	Rat		Blood pressure and heart rate	Höcht, Opezzo, & Taira, 2005b.
Kynurenic acid,		Gerbils	Glu		Moroni et al., 2003.
LCCG1, LAP4	Spinal dorsal horn	Monkey		Extracellular recordings	Neugebauer, Chen, & Willis, 2000.
L-NMA, SC-560, NS-398	Hind paws	Rat	NO, PGE2, and 6-keto-PGF1alpha		Toriyabe et al., 2004
LSD	Prefrontal cortex	Rat	Glu		Muschamp et al., 2004.
Mecamylamine, atropine, SCH22390, eticlopride	Prefrontal cortex, medial temporal cortex, dorsal hippocampus, ventral hippocampus	Rat	MHPG, NA, DOPAC, DA, 5-HIAA, HVA, 5-HT		Rossi, Singer, Shearman, Sershen, & Lajtha, 2005
Methylmercury	Striatum	Rat	DA		Faro et al., 2005.
Metoprolol	Anterior hypothalamus	Rat	DOPAC and 5HIAA	Blood pressure and heart rate	Höcht et al., 2005a.
NMDA	Lateral hypothalamus	Rat		Feeding, behavioral analysis	Duva et al., 2001
NMDA	Forebrain	Chick	Taurine, 5-HT, 5-HIAA	Extracellular recordings	Gruss, Bredenkotter, & Braun, 1999.
NMDA, SC560, NS398	Dorsal hippocampus	Rat	PGE2, 15-F2t-IsoP		Pepicelli et al., 2005
Paroxetine, GR205171	Dorsal raphe nucleus	Mice	5-HT		Guiard et al., 2004.
Phencyclidine	Ventral hippocampus	Rat	cAMP		Klamer et al., 2005.
Raclopride	Nucleus accumbens	Rat	Adenosine		Nagel & Haube, 2004.
SB 206553	Striatum and prefrontal cortex	Rat	DA		Alex, Yavanian, McFarlane, Pluto, & Pehek, 2005.
Vasopressin	Medial preoptic area	Rat	Luteinizing hormone		Palm, van der Beek, Wiegant, Buijs, & Kalsbeek, 2001.
Verapamil, probenecid	Hippocampus	Rat	5-HT, DA		Clinckers, Smolders, Meurs, Ebinger, & Michotte, 2005.
β-phenylethylamine	Ventral tegmental area	Rat	DA		Ishida et al., 2005.

Abbreviations: 15-F2t-IsoP: 15-F2t-isoprostane, 5-HIAA: 5-hydroxyindole acetic acid, 5-HT: serotonin, 8-OH-DPAT: 8-hydroxy-2-(Di-*n*-propylamino)tetralin, ACh: acetylcholine, ACPD: (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, ASP: aspartate, DA: dopamine, DOI: 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane, DOPAC: 3,4-dihydroxyphenylacetic acid, GABA: gamma-aminobutiric acid, Glu: glutamate, HVA: homovanillic acid, LAP4: L(1)-2-amino-4-phosphonobutyric acid, LCCG1: (2S,19S,29S)-2-(carboxycyclopropyl)glycine, L-NMA: *N*-monomethyl-L-arginine acetate LSD: Lysergic acid diethylamide, MHPG: 3-methoxy-4-hydrophenylglycol, NA: noradrenaline, NMDA: *N*-methyl-D-aspartate, NO: nitric oxide, PDC: L-*trans*-pyrrolidine-2,4-dicarboxylic acid, PGE2: prostaglandin E2.

Table 3
Overview of recently published studies using the reverse microdialysis technique in clinical studies

Drug	Subjects	Tissue	Analytical determination	Reference
Metformin	Obese, hyperinsulinaemic, hypertensive subjects	Abdominal subcutaneous adipose tissue	Glycerol, lactate	Flechtner-Mos et al., 1999.
Amrinone	Healthy volunteers	Subcutaneous adipose tissue	Glycerol	Enoksson et al., 1998.
Atrial natriuretic peptide, adrenaline	Healthy volunteers	Subcutaneous adipose tissue	Glycerol	Moro et al., 2005.
Isoprenaline, dobutamine, salbutamol	Obese men	Subcutaneous abdominal adipose tissue	Glycerol	Schiffelers et al., 2003.
Clonidine	Healthy volunteers	Gluteal and abdominal adipose tissue	Glycerol	Galitzky, Lafontan, Nordenstrom, & Arner, 1993.
Isoproterenol	Hypothyroid and Hyperthyroid patients	Abdominal subcutaneous adipose tissue	Glycerol	Nedvidkova et al., 2004.
CGP 12177, terbutaline	Healthy volunteers	Subcutaneous abdominal adipose tissue	Glycerol	Enocksson et al., 1995.
Vanadate	Healthy volunteers	Quadriceps femoris muscle	Glucose, lactate, urea	Hamrin & Henriksson, 2005.
Enalapril	Healthy volunteers	Skeletal muscle	Glucose	Frossard et al., 2000.
Rocuronium, vecuronium	Healthy volunteers	Dermis of the forearm	Protein, histamine, tryptase and bradykinin	Blunk et al., 2003.

coperfusion of naloxone. These results suggested the existence of an opioid-induced inhibition of tonic GABA release in the periaqueductal gray matter, which may in turn lead to a disinhibition of descending pain inhibitory (Stiller et al., 1996). So, local administration of analgesic drugs at different central nuclei by reverse microdialysis allows the elucidation of pain neurotransmission.

Study of dopamine (DA) release by psychostimulants drugs by reverse microdialysis allows a better understanding of the mechanism of action of drugs of abuse. DA released by drugs of abuse is clearly involved in positive reinforcement, particularly in the nucleus accumbens, where it is necessary to reward approach and operant behavior (Wise, 1989). Amphetamine (Hernandez, Lee, & Hoebel, 1987) or cocaine (Hernandez & Hoebel, 1988) given by means of the microdialysis probe significantly increased DA and serotonin (5-HT) release. The ability of hallucinogens to increase extracellular glutamate in the prefrontal cortex was also assessed by in vivo microdialysis. Lysergic acid diethylamide induced an increase in prefrontal glutamate levels, which was blocked by a 5-HT_{2A} antagonist (Muschamp, Regina, Hull, Winter, & Rabin, 2004). Moreover, a phenethylamine hallucinogen, the 5-$HT_{2A/C}$ agonist [−]-2,5-dimethoxy-4-methylamphetamine have similar effects with regards to lysergic acid. The authors concluded that an enhanced release of glutamate is a common mechanism in the action of hallucinogens. Taking together, reverse microdialysis permits the study of the mechanism of drug reinforcement of different drugs of abuse.

Another application of reverse microdialysis in the central nervous system is the study of the mechanism of action of diverse therapeutic agents. Reverse microdialysis experiments also allow the study of other drug effects in an independent way to microdialysis technique. It is possible to monitor the cardiovascular response, the electrical activity at the site of microdialysis, behavioral responses, seizure activity, and body temperature (Table 2).

In a previous study, Höcht, Opezzo, and Taira (2005a) evaluated the possible hypothalamic antihypertensive effect of metoprolol and its action on aminergic neurotransmission in sham operated and aortic coarctated hypertensive rats using the reverse microdialysis technique. Intrahypothalamic perfusion with metoprolol induced a significative decrease of blood pressure in coarctated hypertensive animals but not in normotensive rats. On the other hand, metoprolol perfusion reduced hypothalamic levels of DOPAC in hypertensive rats but not in sham operated animals. Therefore, the authors concluded that the hypotensive effect of metoprolol perfusion in aortic coartated rats suggest that hypothalamic β-adrenergic blockade is part of the antihypertensive effect of metoprolol in chronic coarctated rats (Höcht et al., 2005a). Local administration of antihypertensive drugs by reverse microdialysis in central nuclei involucred in blood pressure regulation allows elucidating the mechanism of action of this therapeutic class.

Studies combining microdialysis and electroencephalography have investigated the mechanism of seizure induction and treatment in various brain nuclei (Bourne, 2003). The site of action of ethosuximide was studied by means of reverse microdialysis and simultaneous electroencephalogram recording (Richards et al., 2003). Ethosuximide was administered into the thalamic nuclei through the microdialysis probe in a genetic rat model of absence seizures. Administration of the anticonvulsivant induced a significant but delayed reduction of spike and wave discharges on the electroencephalogram, suggesting that targeting of the thalamus alone may be insufficient for an immediate and full anti-absence action for ethosuximide (Richards et al., 2003).

In addition, effects of reverse microdialysis of *N*-methyl-D-aspartate (NMDA) into the lateral hypothalamus on feeding and other behaviors were studied (Duva et al., 2001). It was observed that NMDA perfusion induced a stimulation of feeding without a concomitant hyperactivity, supporting a role for

glutamate neurotransmission in the lateral hypothalamus in the control of feeding.

Ishiwata et al. (2004) studied the possible role of serotoninergic neurotransmission in the preoptic area and anterior hypothalamus in regulating body temperature. For this purpose, the authors perfused via microdialysis fluoxetine and 8-OH-DPAT into the preoptic area and anterior hypothalamus and simultaneously evaluated the body temperature and 5-HT and 5-hydroxyindole acetic acid (5-HIAA) dialysate levels. Although, perfusion of both drugs induced changes in 5-HT and 5-HIAA dialysate levels, no changes in body temperature were found, suggesting that serotoninergic neurotransmission in the preoptic area and anterior hypothalamus may not mediate acute changes in thermoregulation (Ishiwata et al., 2004).

The reverse microdialysis technique could also be used for the study of *p*-Glycoprotein-mediated efflux of drugs at the blood–brain barrier. It was found (Potschka, Fedrowitz, & Löscher, 2002) that local perfusion of verapamil, a *p*-Glycoprotein inhibitor, through the microdialysis probe increased the concentration of phenobarbital, lamotrigine, and felbamate in the extracellular fluid of the cerebral cortex in a significant manner. Therefore, these anticonvulsivant drugs are substrate for multidrug transporters at the blood–brain barrier and coadministration with multidrug transporter inhibitors significantly potentiates the anticonvulsant activity of oxcarbazepine. So, reverse microdialysis is a promising methodology for the study of the mechanisms of pharmacoresistance of central acting drugs.

Finally, the protective effect of thiol group donating compounds on methylmercury toxicity at the central nervous system was studied by means of reverse microdialysis experiments in the striatum of rats (Faro et al., 2005). Pretreatment with glutathione, cysteine and methionine decreased dopamine release induced by intrastriatal perfusion of methylmercury. Therefore, administration of compounds containing free thiol groups prevented the methylmercury-induced dopamine release from rat striatum (Faro et al., 2005).

6.1.2. Reverse microdialysis of other tissues

Shyr, Chen, Lu, and Tan (1999) demonstrated the possibility of using reverse microdialysis for dynamic monitoring of hepatic metabolic function. Reverse microdialysis was done by implanting a microdialysis probe into the middle lobe of the liver; the probe was then perfused with a lidocaine-containing solution. Concentrations of lidocaine and its major metabolite, monoethylglycinexylidide, were measured in the dialysate. Metabolic ability was assessed by dividing the monoethylglycinexylidide production by lidocaine administration.

Perfusion of drugs through the microdialysis probe implanted in the sinoatrial node allows the study of drugs actions on heart rate. Farias, Jackson, Stanfill, and Caffrey (2001) found that the opiate receptors responsible for the inhibition of vagal bradycardia are located within the sinoatrial node with few, if any, participating extra-nodal or ganglionar receptors. Moreover, perfusion of the sinoatrial node of dogs with endogenous opioid compounds induced a bradychardic response by activation of δ2-opioid receptors located in the sinoatrial node, supporting evidence of the existence of vagotonic opioid receptors in the sinoatrial node (Farias, Jackson, Yoshishige, & Caffrey, 2003).

The mechanism of inflammation and nociception was studied in the rat hindpaw by means of reverse microdialysis. Local administration of amitriptyline into the rat hindpaw produced an increase in extracellular levels of adenosine, suggesting a possible role for adenosine in the peripheral antinoceptive actions of amitryptiline (Sawynok, Reid, Liu, & Parkinson, 2005). In another report (Toriyabe, Omote, Kawamata, & Namiki, 2004), N-nitro-l-arginine methyl ester (L-NAME) perfusion in the rat hindpaw supressed cyclooxygenase (COX-2) up-regulation induced by carrageenan, indicating that nitric oxide up-regulates COX-2 expression in the late phase of skin inflammation, which would contribute to exacerbation of the inflammatory process.

Reverse microdialysis was also employed for the study of vasoactive substance on corpora lutea growth of the cow. Kobayashi et al. (2002) have found that perfusion of the corpora lutea with angiotensin II stimulated substance-P release, but exposure to atrial natriuretic peptide enhanced angiotensin II release in the corpora lutea.

6.2. Clinical studies

In the clinical setting reverse microdialysis has been used for the study of local effects of drugs in the adipose tissue, skeletal muscle and dermis. A summary of recent studies of reverse microdialysis in clinical research is given in Table 3. The most relevant findings of these studies are discussed below.

6.2.1. Lipolytic drugs

Reverse microdialysis was extensively employed for the study of lipolytic and anti-lipolytic drugs. Microdialysis has some advantages with regard to in vitro lipolysis studies because a particular study reflects the metabolism of adipose tissue in its natural environment and microdialysis offers the possibility to perform local manipulation and simultaneously study metabolism (Kolehmainen et al., 2000). However, in vivo microdialysis recovery is dependent on local tissue blood flow. To eliminate this variability, blood flow must be simultaneously measured using ethanol dilution technique (Kolehmainen et al., 2000). Ethanol is added to the perfusion media and ethanol concentrations were analyzed from the perfusate and dialysate. Changes in the concentrations of ethanol expressed as dialysate/perfusate ratio describe the changes in the local blood flow (Kolehmainen et al., 2000). Lipolytic effects are determined by the measurement of glycerol dialysate concentrations previously and during administration of the lipolytic or anti-lipolytic drug through the microdialysis catheter.

Another advantage of the use of reverse microdialysis for the study of drug effects on lipolysis is the fact that this technique allows the evaluation of the concentration–response relationship in a single subject by perfusing the microdialysis catheter with different concentration of the lipolytic agent. Schiffelers, Akkermans, Saris, and Blaak (2003) studied the relationship between the dose and the lipolytic action of three beta-

adrenergic agonist, salbutamol, isoprenaline and dobutamine, in obese and lean men. The authors found that the lipolytic response to β_1-, β_2- and nonselective β-adrenergic stimulation in situ is comparable in lean and obese male subjects.

In different reports, the lipolytic actions of diverse drugs such as phosphodiesterase 3 inhibitors (Enoksson, Dagerman, Hagström-Toft, Large, & Arner, 1998), atrial natriuretic peptides (Moro et al., 2005) and β-adrenergic agonists (Enocksson, Shimizu, Lonnqvist, Nordenström, & Amer, 1995; Schiffelers et al., 2003). The antilipolytic effects of metformin (Flechtner-Mos, Ditschuneit, Jenkinson, Alt, & Adler, 1999) and α_2-adrenergic agonist (Galitzky, Lafontan, Nordenstrom, and Arner, 1993) were also measured by means of in situ microdialysis (Table 3).

The antilipolytic effect of metformin was investigated in obese, hyperinsulinaemic, hypertensive subject by means of reverse microdialysis (Flechtner-Mos et al., 1999). For this purpose, the authors determined glycerol and lactate dialysate concentrations in the presence of metformin and after administration of catecholamines using microdialysis. It was found that metformin lowered glycerol release and suppressed the lipolytic activity of catecholamines (Flechtner-Mos et al., 1999).

In another report, Nedvidkova et al. (2004) demonstrated that thyroid function affects the lipolytic effects of beta-adrenergic agonists. Hypothyroidism resulted in attenuated and hyperthyroidism in enhanced, lipolytic response to local stimulation with isoprenaline administered by reverse microdialysis.

6.2.2. Muscle metabolism

Reverse microdialysis technique was used for the study of therapeutic drug effects on muscle metabolism. So, the local effect of the insulin-mimetic agent vanadate on glucose metabolism in human skeletal muscle was studied using the microdialysis technique (Hamrin & Henriksson, 2005). Vanadate was locally administered through the microdialysis catheter and simultaneously glucose and lactate were collected in the dialysate. It was found that vanadate decreased interstitial glucose concentrations and increased lactate levels, in the vicinity of the microdialysis catheter, indicating that vanadate mimics the effect of insulin in human skeletal muscle in vivo (Hamrin & Henriksson, 2005).

The mechanism of insulin sensitivity improvement by angiotensin-converting enzyme inhibitors was studied by means of microdialysis in skeletal muscle (Müller et al., 1997). Selective inhibition of paracrine angiotensin converting-enzyme activity by enalapril increases glucose and lactate interstitial concentrations and decrease serum interstitial gradient of glucose in muscle by facilitating transcapillary glucose transport explaining the improved insulin sensitivity to enalapril (Müller et al., 1997).

6.2.3. Skin adverse drug reactions

Adverse drug reactions at the dermis could also be studied by cutaneous reverse microdialysis sampling of endogenous compounds like histamine, prostaglandins, tryptase and bradykinin. It is well known that rocuronium and vecuronium can induce burning sensations associated with withdrawal reactions during administration. So, dermal microdialysis in humans was used to elucidate the underlying mechanisms of pain induction (Blunk, Seifert, Schmelz, Reeh, & Koppert, 2003). Microdialysis catheters were inserted intradermally and perfused with rocuronium and vecuronium. Dialysate were analyzed for protein, histamine, tryptase and bradykinin content. Rocuronium induced sharp burning pain, whereas vecuronium given in the usual clinical concentration induced only minor pain sensations. No correlations were found between pain rating and mediator release, concluding that the alogenic effect of neuromuscular blocking drugs is not related to release of algesic mediators (Blunk et al., 2003).

Reverse microdialysis could also be employed for the design of experimental models of dermis inflammation. A comparison of intradermal injections with an atraumatic intraprobe drug delivery system for codeine-induced histamine release in intact human skin was made (Petersen, Nielsen, & Skov, 1995). Although, peak histamine release was found within the first 4 min after skin challenge by intraprobe drug delivery system and intracutaneous injections of codeine, the coefficient of variation on peak histamine release was lower with intraprobe drug delivery compared to intracutaneous injection. Therefore, the authors concluded that codeine could be atraumatically administered to the skin by intraprobe delivery and it would be possible to deliver immunopharmacologically active drugs to the skin by intraprobe delivery (Petersen et al., 1995).

6.3. In vitro application of reverse microdialysis

Reverse microdialysis was also used for the perfusion of different compounds in in vitro preparations. So, Acosta et al. (1999) studied the local interrelationships among angiotensin II, endothelin and atrial natriuretic peptide and the possible effect of these compounds on the secretion of steroid hormones and prostaglandins in isolated bovine mature follicles. For this, four microdialysis probes were implanted in isolated preovulatory follicles and perfused with vasoactive compounds. The authors found that perfusion of endothelin induced the release of atrial natriuretic peptide and estradiol, but diminished androstenedione and progesterone levels. Angiotensin II stimulated the release of endothelin, progesterone and estradiol, but inhibited atrial natriuretic peptide release. The results demonstrated a complex interaction among angiotensin II, endothelin and atrial natriuretic peptide that may contribute to increasing the follicular production of prostaglandins and modulate steroidogenesis in the mature follicle. In another study, Wijayagunawardane et al. (2001) used an in vitro microdialysis system in the oviduct for the simultaneous study of oviductal contraction and secretion of prostaglandins and endothelin during perfusion of the microdialysis probe with luteinizing hormone, steroids, prostaglandins and peptides. Perfusion of the oviduct with luteinizing hormone alone or combined with progesterone, estradiol-17β or endothelin stimulated release of different prostaglandins. On the other hand, reverse dialysis of

luteinizing hormone increased the amplitude of oviductal contraction.

7. Reverse microdialysis as a therapeutic tool

A perspective of reverse microdialysis is the use of this technique as a therapeutic tool. Ronquist, Hugosson, Sjolander, and Ungerstedt (1992) successfully employed reverse microdialysis of antineoplasic drugs for site specific drug delivery in the treatment of gliomas. 3 patients with inoperable malignant glioma were treated by direct and continuous administration of L-2, 4 diaminobutyric acid in tumor tissue employing reverse microdialysis for a total of 14–21 days without side effects assignable to the drug. L-2, 4 diaminobutyric acid administered through the microdialysis catheter into malignant brain tumor tissue was well tolerated and showed promising antitumour activity. However, there are not recently studies that employ the reverse microdialysis technique for local drug administration to treat diseases. The applicability of reverse microdialysis as a therapeutic tool is hampered by several factors. Microdialysis in patients must be conducted in strict compliance with regulatory demands and need to be based on appropriate ethical conditions. On the other hand, microdialysis probe implantation produces some discomfort and sleep disturbance. Finally, a limitation of the use of reverse microdialysis as a therapeutic tool is the generation of tissue alteration by chronic probe implantation that affects the microdialysis catheter performance.

8. Conclusions

Reverse microdialysis is a unique and powerful technique for the study of pharmacological and toxicological agents, because it allows the local delivery of therapeutic agents and simultaneously monitoring its effect on endogenous compounds and other physiological parameters. In basic research, the main application of reverse microdialysis is the study of local effects of drugs on neurotransmission. Reverse microdialysis also allows the study of drug actions on lipolysis, muscle metabolism and the dermis in healthy volunteers and patients. The mayor drawback of the reverse microdialysis technique is the cost of the equipment and the low time resolution, limiting its use in behavioral studies.

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