ELSEVIER

Journal of Chromatography B, 747 (2000) 21–32

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Analysis of biogenic amines in microdialysates of the brain

Ben H.C. Westerink*

Department of Biomonitoring and Sensoring, Deusinglanan 1, 9713 AN Groningen, The Netherlands

Abstract

Microdialysis is a method of sampling a liquid compartment by means of a hollow fibre dialysis membrane. The method was developed in the 1980s as a technique for sampling the extracellular fluid of the brain of conscious animals. When used in combination with sensitive analytical chemical tools, microdialysis can be used to study the regulation of neurotransmission in the living brain. Here we describe the application of microdialysis for sampling and detection of biogenic amines (dopamine, noradrenaline and serotonin) in brain tissue. A short overview of the microdialysis technique and its applications are given. In addition, the analytical chemical methods that are currently used to assay biogenic amines in dialysates are briefly discussed.

Keywords: Reviews; Biogenic amines

Contents

1. Introduction 22
2. Methods of microdialyis 22
   2.1. Cannula 22
   2.2. Perfusion 23
   2.3. Recovery 23
   2.4. 'True' extracellular concentration of biogenic amine transmitters 24
3. Validity and limitations 24
   3.1. Recovery period 24
   3.2. Removal of endogenous molecules 25
   3.3. Limited time resolution 25
   3.4. Are the sampled neurotransmitters derived from neurons? 25
4. Applications 26
   4.1. Pharmacology 26
   4.2. Study of animal behaviour 27
   4.3. Interactions in the brain 27
5. Analytical chemistry of biogenic amines 27
   5.1. History 27
   5.2. Sample clean-up 28
   5.3. HPLC and electrochemical detection 28
      5.3.1. Pumps 28

*Tel.: +31-50-363-3307; fax: +31-50-363-6908.
E-mail address: westerink@farm.rug.nl (B.H.C. Westerink).

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00338-8

5.3.2. Columns 28
5.3.3. Mobile phases 28
5.3.4. Detectors 28
5.3.5. Detection limits 29
5.4. Other methods 29
6. Future developments 29
References 31

1. Introduction

Microdialysis is a powerful and versatile sampling technique by which endogenous compounds are continuously monitored in the extracellular fluid of living organisms. The method is based on the dialysis principle in which a hollow fibre, that is permeable to water and small molecules, separates two fluid compartments. During perfusion of the fibre, molecules are exchanged by diffusion in both directions. Microdialysis in the present form was introduced by Johnson, Ungerstedt and co-workers in 1983 [1,2].

Microdialysis originated in neuroscience laboratories as a method to sample the extracellular fluid of living brain tissue. It is now a firmly established method for monitoring the release of dopamine, noradrenaline and serotonin in the brain of conscious animals. Several reviews [3–9] and a monograph [10] provide a comprehensive overview of the recent developments in microdialysis methodology. The impact of the method is well illustrated by the fact that more than 5000 articles reporting use of the method have appeared since 1983. About 50% of these articles concern biogenic amines.

Brain microdialysis is often carried out on rodents, usually rats, but also mice, guinea pigs, cats and monkey are used. Sheep are excellent experimental animals for microdialysis as battery-driven syringe pumps are attached to the wool on the animals' back [11]. Although brain microdialysis is now a routine technique in certain surgery units in hospitals, we are not aware of clinical studies on biogenic amines.

As the limits of the microdialysis method are usually set by the sensitivity of the assay techniques, it is evident that the development of analytical chemical methods was, and is, of great importance for the impact of microdialysis of biogenic amines.

2. Methods of microdialyis

2.1. Cannula

The microdialysis cannula (called the probe) typically consists of a tubular dialysis fibre (diameter: 0.2–0.3 mm) that is connected with a rigid inlet and outlet tube. The fibre is constantly perfused with a physiological fluid. To allow the animal a freely moving behaviour, the inlet and outlet tubes are connected with thin and flexible tubing to the perfusion pump, autoinjector or HPLC equipment. To facilitate the behaviour of the animal, a swivel is often used.

Although microdialysis cannulas are commercially available in various dimensions, many laboratories have designed their own probe as its construction lasts not longer than a few minutes. Various designs have been described; examples of probes are shown in Fig. 1. Hollow dialysis fibres (derived from artificial kidneys) are glued by means of epoxy resin to stainless steel or polyethylene tubing providing an inlet and an outlet. The length of the fibre is adjusted to the brain region that is sampled. The part of the fibre that is in contact with brain tissue, but not aimed to be perfused, is covered by glue.

The transcerebral probe (Fig. 1) results in a high recovery and relative few damage to the brain tissue. However its implantation causes extensive damage to skin and muscles and not every brain region can be sampled by this approach. The I-shaped probe is currently much used. Because of its narrow cylindrical shape the I-shaped probe can be handled like a needle. Using stereotaxic surgery almost any brain region can be sampled. The minimal length of the membrane that is exposed is between 0.5 and 1 mm, which means that also the small monoaminergic nuclei in the (rat) brain, such as the substantia nigra,

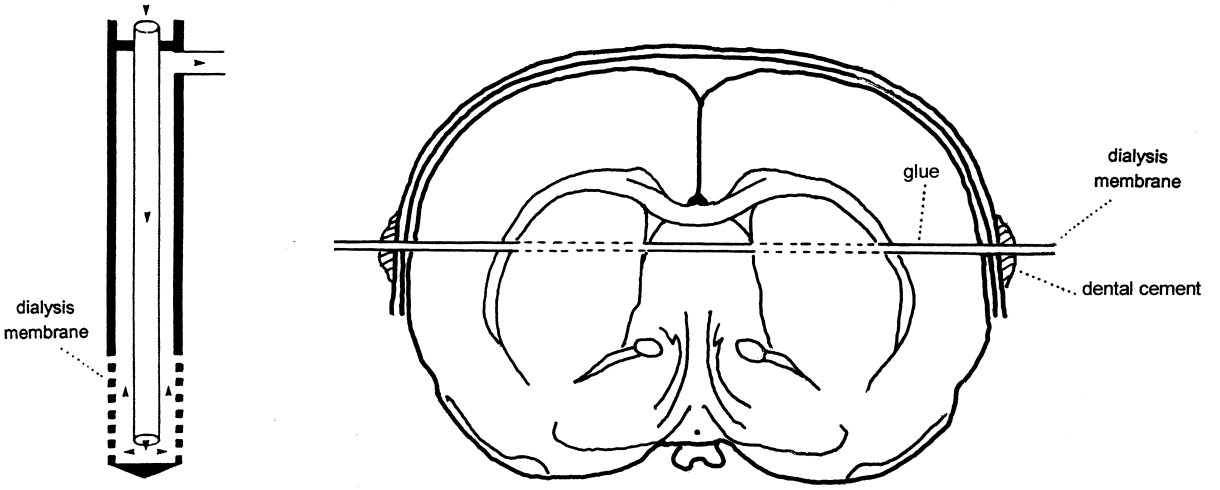

Fig. 1. Two examples of microdialysis probes: the vertical I-shaped probe (left), and the transcerebral probe (right) implanted bilaterally in the striatum of the rat.

nucleus accumbens, nucleus raphe, locus coeruleus etc., can be sampled reproducibly.

A wide rage of kidney dialysis fibre types has been employed to construct microdialysis probes. Cellulose, polycarbonate–ether or polyacrylonitrile–sodium methallylsulfonate (Hospal) polymers are often used. Although the Hospal fibres have high recovery rates, the penetration rate for serotonin is slow because of the fixed negative ionic charge on the surface of the fibres [12]. In addition it was found that certain lipophilic drugs have difficulties in passing the Hospal membrane (unpublished observations in our laboratory).

2.2. Perfusion

As a rule perfusion fluids are isoosmolar and contain the relevant physiological ions. The perfusion fluid not only extracts neurotransmitters from the extracellular fluid, but all kinds of small molecules and ions are continuously removed from brain tissue and this may disturb the homeostatic balance of the extracellular environment. It is therefore essential to keep the ion composition of the perfusion fluid as close as possible to that of the brain interstitial fluid.

Microdialysis samples often contain around 0.1–2 pg of neurotransmitter, which is close to the detection limit of the current analytical methods. Various workers therefore routinely add pharmacologically active compounds to the perfusion fluid to facilitate the analytical chemical detection. Monoamine reuptake inhibitors and additional Ca^{2+} (2.4 instead of 1.2 mmol) are commonly used in this respect. The question arises of course, as to whether these additions create artificial conditions. If a serotonin reuptake inhibitor is included in the perfusion fluid, systemic administration of the same inhibitor results in a decrease, rather than the expected increase, in dialysate serotonin in nerve terminal area [13]. Selective stimulation of somatodendritic autoreceptors is believed to be responsible for this effect. Although such data provide interesting information about the possible tonic activation of the various (auto)receptors during in vivo conditions, it is emphasised that addition of calcium or reuptake inhibitors to the perfusion fluid, only because of the limited sensitivity of the available analytical chemical techniques, should be prevented, if possible.

2.3. Recovery

The recovery (often called the extraction-factor) of the sampled transmitter depends on the properties of the membrane, the size of the molecule (in terms of molecular mass and lipophilicity) and the flow-rate of the perfusion. When flow-rates around 1–2 μl/

min are applied, recoveries around 20% are to be expected for dopamine, noradrenaline and serotonin. Higher recoveries (close to 90%) can be reached when flow-rates are around 0.1–0.5 μl/min, however these rates are not always practically as they result in long latency times in the tubing.

At flow-rates between 2 and 0.5 μl/min, the relative recovery of sampled compounds (expressed in concentration units) decreases when the flow-rate increases, whereas the absolute recovery (amount per total sample) is virtually constant (review in [8,9]). This means that when the total sample is injected into an HPLC system, the perfusion flow-rate hardly contributes to the output of the sampled transmitter.

Low recovery is often considered to be a weak point of the microdialysis technique, however, it can also be considered advantageous as a limited extraction of endogenous compounds will have less disturbing effects on the homeostasis of the brain.

2.4. 'True' extracellular concentration of biogenic amine transmitters

Several approaches have been proposed to calculate the true extracellular concentration of biogenic amine transmitters in extracellular fluid hat surrounds the membrane. Methods that aim to eliminate concentration gradients around the probe, such as the 'zero net flux' method [14] and 'extrapolation to zero flow' [15], have been proposed. These approaches are based the assumption that extraction and recovery rates are equal quantities [16]. In a recent study in which microdialysis probes and voltammetric electrodes were compared, it was demonstrated that this assumption is not valid for dopamine [17]. Recovery of dopamine in latter experiments was initially near zero and increased upon uptake inhibition, suggesting that the no-net-flux method underestimate the basal extracellular concentration of dopamine.

Because of these uncertainties several workers have emphasised that microdialysis is a relative sampling technique of which the results should be expressed and interpreted in terms of relative changes (percentage of controls) [7,8]. Given the fact that the relative recovery for biogenic amines depends on uptake rates, such approach is only valid when a constant recovery can be assumed. This assumption was evaluated for dopamine. It appeared that changes in extracellular dopamine recorded during various behavioural conditions (e.g. feeding, novelty, handling) were similar in the absence or presence of a reuptake inhibitor [18,19].

3. Validity and limitations

3.1. Recovery period

Microdialysis is an invasive technique and will always cause substantial damage to nerve tissue. During the first hours after probe implantation several processes are affected. There is a decreased blood flow in the tissue surrounding the probe, a reduced oxygen uptake, an abnormal release of neurotransmitter and the integrity of the blood–brain barrier might be disturbed [9,20–22]. However, 24 h after implantation of the probe these processes appear to have recovered substantially [23]. Three days after implantation a glial barrier around the cannula tract has been described [4]. The development of gliosis often limits the duration of the experiment, as the glial barrier is probably responsible for the fact that the extracellular levels of neurotransmitters gradually decrease 3–4 days after implantation [8]. Microdialysis experiments are therefore limited in time and last in general not longer than several days. Much — but not all — of this interference might be overcome by the use of a guide cannula in which a removable probe is inserted when the animal is sufficiently recovered from the surgery.

For pharmacological studies a recovery period for the surgery of about 24 h is often considered satisfactory. However for behavioural studies it is questionable whether this period is sufficient. Telemetric recordings demonstrated a pronounced effect of the surgery on both amplitude and rhytmicity of the temperature and activity patters, up to 6–7 days after probe implantation [24]. Again guide cannulas are recommended to overcome these interferences. Such cannulas allow multiple insertions of the probe, without affecting the behavioural response as was shown for microdialysis recordings during mating in male rat's [25].

The various artefacts that have been observed, do

not mean that no relevant neurochemical or behavioural experiments can be recorded during the first days after implantation of a fixed microdialysis probe. The feeding-induced increase in dopamine release in the nucleus accumbens of food-deprived rats, is already detectable 24 h after implantation of the probe, and this response remains constant for at least 6 days after implantation of a permanent probe [18]. Furthermore it was shown that the circadian rhythm in the release of noradrenaline from the pineal gland, was not affected by the implantation of the probe. Twenty-four hours after surgery a characteristic and very robust circadian rhythm was detected, that was strongly related to the day–night pattern of the animal [26].

3.2. Removal of endogenous molecules

Although the dialysis membrane prevents the removal of large molecules and enzymes, thus minimising perturbation of the neural environment, evidence is provided that during dialysis the surrounding tissue is depleted of crucial constituents such as neurotransmitters and modulators [27,28]. The perfusion itself may induce unphysiological changes in the surrounding brain tissue, which may finally influence the release and metabolism of the sampled transmitter.

3.3. Limited time resolution

Microdialysis samples are usually collected during 5–20 min periods. When the present analytical methods are optimised (e.g. use of microbore columns) sampling times for biogenic amines in the order of 1 min is possible [29]. Although further reduction of the time-resolution is possible (see Section 6), it should be realised that the dimensions of the microdialysis probe are in the millimeter range, which means that the probe collects an integrated chemical signal to which many thousands of neuronal discharges contribute. Although many processes in the brain have time-constants in the order of seconds or milliseconds, it is likely that the time-constant of neurotransmitter changes in dialysates is closer to minutes than to seconds. A practical problem of smaller sampling times is the large amount of samples that is produced.

It is evident that real-time registration of neurotransmitter release, as is achieved by in vivo voltammetry, is difficult to reach with the microdialysis method.

3.4. Are the sampled neurotransmitters derived from neurons?

Microdialysis does not sample neurotransmitter directly from the synaptic cleft, but the method detects the compound relatively far away from the site were it is released. As the transmitter diffuses away from the synaptic cleft, changes in concentration will be attenuated and diluted by processes such uptake mechanisms, enzymatic degradation and tortuosity. In case of biogenic amines it has been emphasised [30] that uptake mechanisms play a crucial role in determining the final concentration in the extracellular fluid.

The central question is whether neurotransmitter levels in dialysates reflect true synaptic release or more unspecific overflow from non-synaptic sources. A classical feature of neuronal activity is membrane depolarisation that is initiated by the opening of fast Na^+ channels to allow influx of Na^+ ions. In addition there is ample evidence that biogenic amines are released from vesicles by exocytosis, a process which is calcium-dependent. Both the dependency of sodium-channel and the calcium-dependency of the sampled biogenic amine can be evaluated during microdialysis experiments [22,23,31]. For the involvement of sodium-channels, the channel blocker tetrodotoxin (TTX) is infused by retrograde dialysis, whereas calcium-dependency is determined by omitting calcium from the perfusion fluid or by infusing calcium-channel blockers.

The TTX-dependency of biogenic amines sampled by microdialysis has been determined in numerous studies. Most studies agree that these transmitters respond to TTX infusion by a rapid (5–15 min) and pronounced disappearance (>90%) from the dialysate samples [3,23]. In the case of dopamine it has been shown that in acutely implanted animals the TTX-dependency of the neurotransmitter output is less pronounced than 8–24 h after implantation [23]. This suggests that in acute experiments a certain fraction of damaged nerve terminals might contribute to the sampled neurotransmitter level. By studying

four different probes it was demonstrated [32] that during the first hours after implantation, the traumatic disruption of neurons — reflected by insensitivity to TTX — is directly dependent on the diameter of the probe. However 24 h after implantation, all types of probes displayed a fully TTX dependent release of dopamine. Because of the efficacy of TTX, the compound is used in many laboratories not only as a tool to demonstrate physiological release, but also as a final proof of the identity of the transmitter in the chromatogram.

In several studies the Ca^{2+} dependency of the neurotransmitters content in dialysates has been investigated [22,31]. Omitting Ca^{2+} from the perfusion fluid usually reduces the dialysed levels of the biogenic amines by 70–80%. However the microdialysis probe cannot remove the Ca^{2+} ions completely from the surrounding extracellular fluid, which means that a total inhibition of neurotransmitter release is difficult to achieve. An almost complete depletion of transmitter levels in dialysates is reached when calcium-blocking ions such as Cd^{2+} or high concentrations of Mg^{2+} are included in the perfusion media [31]. Addition of calcium chelating agents such as EDTA to the perfusion medium has also been used to demonstrate calcium-dependency of transmitter release in dialysates.

It can be concluded from TTX- and calcium-depletion studies, that the major part of dopamine, noradrenaline and serotonin present in brain dialysates is derived from action-potential controlled release. Here the biogenic amines clearly differ clearly from the amino acid neurotransmitters glutamate and GABA, which display no or only a limited calcium- and TTX-dependency in microdialysates [33].

4. Applications

Microdialysis of biogenic amines is now a routine technique in neuroscience laboratories. Numerous applications have been described for neuropharmacological as well as behavioural research. Here only some typical examples are given. They are biased by the contribution of the author's own laboratory.

4.1. Pharmacology

Known and experimental centrally-acting drugs have been frequently investigated by the microdialysis technique. Although drugs are often systemically administered, specific information is obtained when a compound is directly administered to the target site by local application (called retrograde dialyses). With this approach receptor-specific interactions can be studied in detail. Presynaptic autoreceptors are known to play an important role in the regulation of the release and synthesis of several neurotransmitters including the biogenic amines [34,35]. The microdialysis technique has provided detailed information about the properties of autoreceptors in vivo.

Based on microdialysis studies our insight into the mechanisms of action of serotonin specific reuptake inhibitors (SSRI) (frequently used as antidepressants) is much improved. Microdialysis provided new information about changes in the release of serotonin after acute as well as chronic administration of SSRIs. It is now evident that inhibitory 5-HT_{1A} serotonin autoreceptors, localised on somatodendritic sites, counteract the SSRI-induced increase in 5-HT release from nerve terminals. Desensitisation of the 5-HT_{1A} receptor on somatodendritic sites is proposed as explanation for the increased release of serotonin that is observed during chronic treatment with reuptake inhibitors. This mechanism would explain the late onset of action of antidepressants. The results of these microdialysis studies have already led to new strategies for the treatment of depression [36,37].

Another interesting field to which microdialysis has contributed is the pharmacology of antipsychotic drugs. Differences between typical and atypical antipsychotic drugs have been evaluated by monitoring the release of dopamine in various brain regions. Studies in frontal cortex, nucleus accumbens and striatum have shown that various antipsychotics display a regional specificity. Moreover interactions of antipsychotics with other receptors (e.g. 5-HT_{2A} and 5-HT_{1A} type) have been studied in detail [38–40].

Nowadays an overwhelming amount of receptors, enzymes and proteins is available, all of them are new interesting targets for medicinal chemists to

develop new drugs. Microdialysis of biogenic amines and other 'classical' neurotransmitters will remain in the future an important tool to detect interactions between newly developed ligands and neurotransmission.

4.2. Study of animal behaviour

There is no doubt that microdialysis is at presently the most versatile and practical method to study the neurochemistry of behaviour [6,7]. Although many types of behaviour have time constants far beyond the sampling time of microdialysis, paradigms such as arousal, reward, stress, conditioning, learning etc., last seconds to minutes, and microdialysis can certainly contribute to a further understanding of these behavioural phenomena.

Microdialysis studies have identified certain brain structures as being particularly involved in reward and stress. The discovery that the nucleus accumbens shell and core are differently involved in feeding behaviour and response to drugs of abuse, have led to new ideas how mesolimbic dopamine neurons are involved in reward and addiction [41,42].

4.3. Interactions in the brain

Interactions in the brain form a major challenge for brain researchers. Biogenic amine systems in the brain are examples of such complex interactions. The various nuclei of the biogenic amines (e.g. nucleus raphe, locus coeruleus and substantia nigra) participate in different types of behaviour such as sleep, attention, arousal, stress, sleep, etc. Moreover these systems are strongly involved in the mechanisms of action of drugs that are used to treat neurological and psychiatric diseases.

Multiprobe microdialysis is a powerful method to study interactions between neuronal systems. Biogenic amine systems are very suitable in this respect, as their nuclei are clustered and often anatomically well separated from the terminal fields. During multiprobe microdialysis receptor specific compounds are infused in certain brain nuclei, whereas remote effects of these infusions are established with a second probe implanted in the nerve terminal area. Used as such, the properties of the receptors present on somatodendritic sites of neurons can be studied and, in addition, postsynaptically induced changes in other neuronal systems can be recorded. Examples of this approach are the dopaminergic projections of the substantia nigra and ventral tegmental area to the forebrain, the serotonergic projections from the nucleus raphe and the noradrenergic projections from the locus coeruleus to the forebrain [43–45].

Dual-probe microdialysis can also be used to further analyse the neurochemistry of behaviour; e.g. during reward or stress receptor specific compounds are infused onto somatodendritic sites of nuclei to evaluate their effect on the behaviour-induced changes in transmitter output. Using this method it could be established that glutamatergic afferents to the ventral tegmental area are involved in reward-related changes in dopamine release from mesocortical dopamine neurones [43,46,47].

5. Analytical chemistry of biogenic amines

5.1. History

The introduction of the electrochemical detector by Kissenger et al. in 1973 [48] and the availability of highly efficient reverse phase columns stimulated the development of sensitive HPLC assays for biogenic amines. Addition of a sulfonated ion-pairing reagent to the mobile phase that converts a reversed-phase column into a strong cation-exchange column, resulted in highly efficient separations. The latter principle, initially called 'soap chromatography' [49,50], was of great value for the chromatography of biogenic amines and related and metabolites. Since its introduction a large number of methods, based on this principle, have been described. Because of the overwhelming amount of published methods, no attempt was made to present here an overview of these studies; only a general (and practical) view is presented.

The availability of assays in the picogram range for biogenic amines, inspired Johnson, Ungerstedt and coworkers in 1983 to apply these methods to samples obtained by dialysis fibres in brain tissue [1,2]. When these attempts were successful, the brain microdialysis technique was born. The method was further developed in several laboratories

[3,5,9,51,52], but it took until the early 1990s before the method was fully introduced into neuroscience.

5.2. Sample clean-up

As the dialysis membrane acts as a filter against the blood cells, proteins and other large molecules present in the ECF, microdialysates are relatively clean and suitable for direct injection into a HPLC system without further purification.

As typical flow-rates used in microdialysis experiments are between 0.5 and 2 μl/min and sample times are often between 1 and 15 min, these conditions will result in small samples that can be easy handled in HPLC systems. It is desirable to analyse microdialysis samples directly on-line producing data in near-real time providing a direct feedback with experiment. Some researchers connect the outlet of the dialysis probe directly to the valve of the HPLC system [3]. With the help of electronically controlled valves, a completely automated semi on-line microdialysis system is then achieved.

5.3. HPLC and electrochemical detection

5.3.1. Pumps

As electrochemical detectors are very sensitive to fluctuations in pressure, HPLC pumps should be used that produce a stable flow-rate. The use of a two-piston HPLC pump, in combination with a pulse dampener is often sufficient to reach the desired low detection limits.

5.3.2. Columns

Silica-based reversed-phase columns (spherical particles of 3 μm, packed in columns of 10–25 cm, with diameters of 4.7, 3 or 2 mm), loaded with sulfonated ion pairing reagent (often heptane or octane sulfonic acid), result in highly efficient separations of biogenic amines (number of theoretical plates around 10 000 per column). As the microdialysis method produces small samples (usually between 0.5 and 50 μl), the use of small bore or microbore columns should be considered. Several groups have described assays with column diameters of 1 or 0.5 mm [29,49,53,54]. Although the use of microbore approach results in an improved detection limit, its use is not generally accepted. The somewhat lower chromatographic performance, the need for additional equipment (adapted valves and electrochemical detector) and the more critical conditions for optimal performance (prevention of dead volumes in connections), results often in an analysis that is more vulnerable and less ‘student proof’ than when traditional columns are used.

5.3.3. Mobile phases

With the currently used buffers (pH range: 3–6), the retention time of the biogenic amines is only slightly pH-dependent, whereas the related acid metabolites such as DOPAC, HVA, 5-HIAA (present in much higher amount in the dialysates than the amines) elute in a strongly pH-dependent manner. At a relatively high pH (>5), acid metabolites of the biogenic amines elute close to the void volume; at lower pH (3–4), the metabolites are strongly retained on the column. The pH of the mobile phase is therefore used to adjust the chromatographic separation of the amines and their metabolites. To obtain reasonable retention times for the biogenic amines, the mobile phase requires a certain amount of organic solvent (often methanol or acetonitrile in the concentration range of 5–15%). When the mobile phase is composed of a relatively high pH (around 6), a strong lipophilic sulfonated ion pairing reagent (e.g. laurylsulphate) and a high methanol/acetonitrile content (about 30%), a complete separation between dopamine, noradrenaline and serotonin can be achieved (the acid metabolites elute close to the void volume) [55]. An example of this mobile phase, developed for rapid and sensitive detection of dopamine, is shown in Fig. 2.

5.3.4. Detectors

BioAnalytical Systems (BAS) was the first company that produced commercially available electrochemical detectors. These detectors are based on thin layer cells, initially filled with carbon paste and later replaced by glassy carbon. Another development was the introduction of the coulometric detector (called Coulochem) by ESA. The Coulochem cell consists of two electrochemical detectors (made from graphite cylinders) that are connected in series. Each detector can either oxidise or reduce the analytes. An interesting application of this cell is the ‘redox mode’, in which compounds are oxidised followed by reduc-

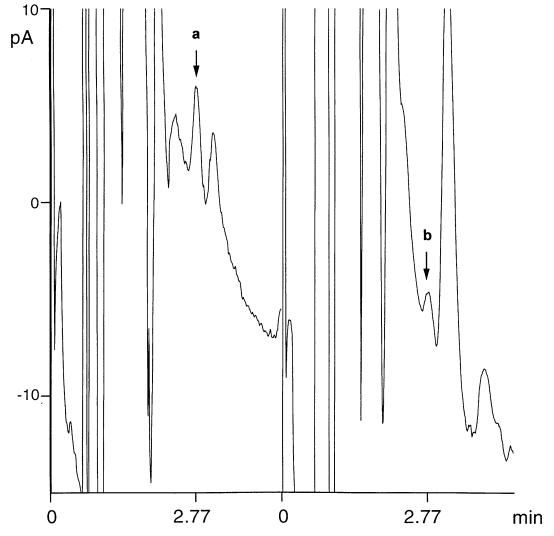

Fig. 2. Four-minute analysis of dopamine by HPLC and ECD. (a): 200 fg (1.3 fmol) dopamine, (b) dialysate of the prefrontal frontal cortex of the rat; collection time: 4 min; laboratory-made concentric probe, Hospal membrane, 2 mm exposed. Chromatographic conditions, mobile phase: Milli-Q water with 0.03 m*M* EDTA, 0.375 g/l citric acid, 3.75 g/l sodium acetate, 450 mg/l sodium lauryl sulphate, 20% methanol and 17%, acetonitrile; pump: Waters 510 with pulse dampener, flow-rate: 0.4 ml/min, injection volume: 5 μl, column: Phenomenex Prodigy (5-μm particles, 100×2 mm, detector: ANTEC Decade with VT-03 flow cell, oxidation at +350 mV (by courtesy of Dr. M.F.P. Feenstra).

tion. With this option reversibly oxidised compounds (such as the catecholamines, but not serotonin) are detected with higher selectivity. The disadvantage of the Coulochem cell is its large internal volume, that prevents its use in small bore (diameter 2 mm or less) applications.

A large electronic and electrostatic stability is achieved by a complete HPLC–ECD system as delivered by ANTEC Leyden (Decade). In this system the thin layer cell, injector, pulse dampener and column are placed in a temperature controlled Faraday cage. Typical results for noradrenaline, dopamine and serotonin in dialysates are shown in Figs. 3 and 4.

5.3.5. Detection limits

It is of great interest to discover which detection limits are reached by the various methods. Detection limits may vary within one laboratory as they depends on quality of the pump, the performance of column, the condition of the electrochemical cell, the electronic stability of the potentiostat and the electrostatic stability of the HPLC system, etc. With conventional columns (2–4.7 mm diameter) detection limits of around 0.2 fmol (5-HT) and 0.5 fmol (dopamine; noradrenaline) in dialysate volumes up to 100 μl, are possible during optimal conditions. In general detectors of BAS and ANTEC display lower detection limits than the ESA coulometric detector.

5.4. Other methods

Radioenzymatic methods have been used to detect noradrenaline in dialysates [56,57]. In addition, several assays based on HPLC with fluorescence detection have been described. Serotonin in microdialysates was quantified by HPLC with fluorescence detection [58]. Here the native fluorescence of the indole group was used to detect serotonin and its metabolite 5-HIAA. Noradrenaline was detected by HPLC and fluorescence detection after precolumn derivatization with diphenylethylenediamine (detection limits up to 1.8 fmol on column of noradrenaline are reported) [59]. Although these assays have low detection limits they were seldom used to detect biogenic amines in dialysates [28], probably because the electrochemical methods reach a sufficient sensitivity.

Recently a HPLC method with fluorescence detection for noradrenaline in microdialysates, based on precolumn derivatization with benzylamine [60], was described. The method was applied for a microbore column (100×1 mm) and the detection limit (signal-to-noise ratio of 3) was reported to be 90 attomol in 10 μl dialysate.

6. Future developments

HPLC–ECD will in the near future maintain its popularity and detectors will be further optimised. When these systems are provided with built in microbore columns and a miniaturised autoinjector, they have the potential to reach the low fg (<100 attomol on column in 10–50 μl dialysates) range, with only minimal maintenance required.

New assays for biogenic amines based on different principles will continually compete with the HPLC–

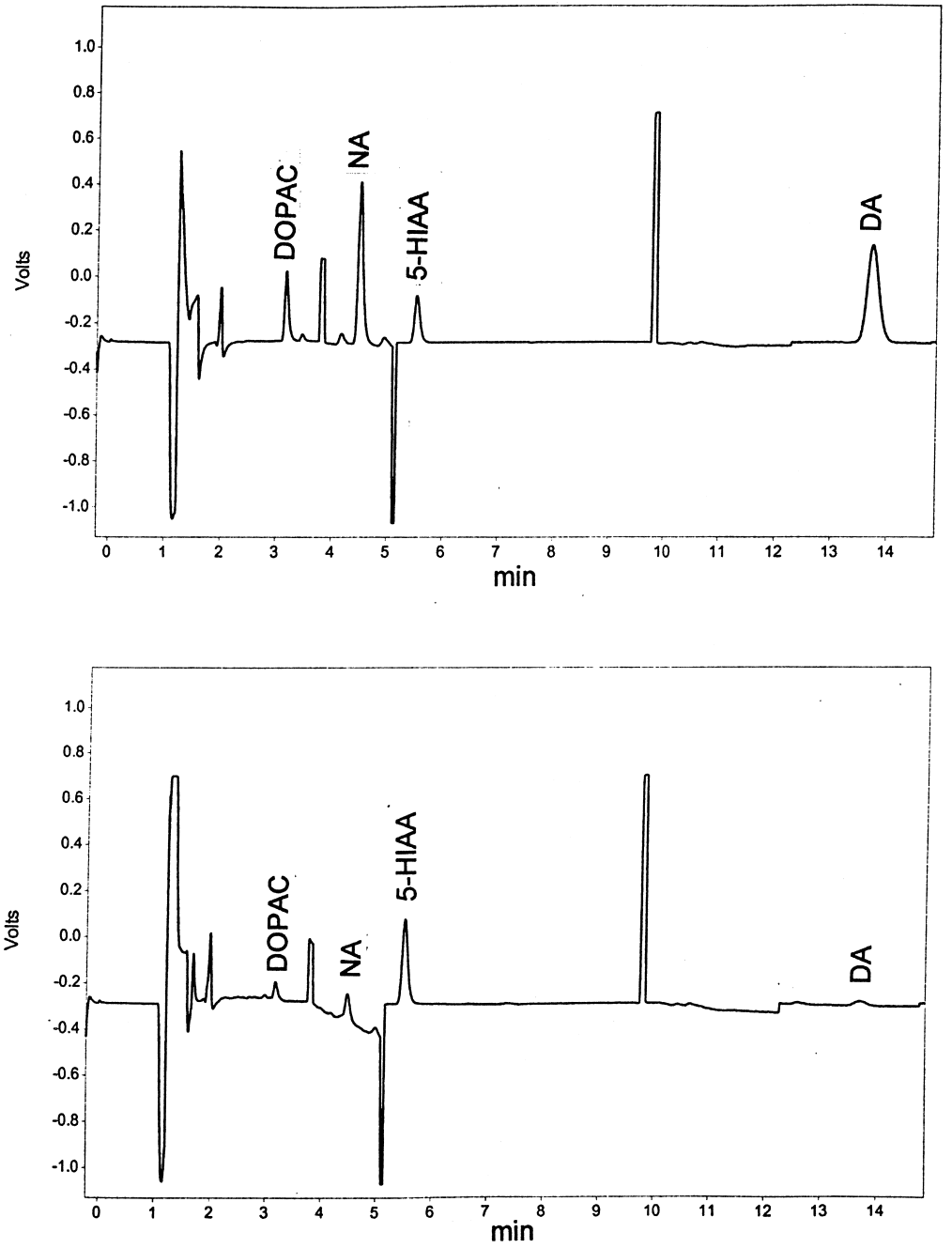

Fig. 3. Dopamine (DA) and noradrenaline (NA) in dialysates of the prefrontal cortex of the rat. Upper trace: standard solution of DA and NE (5 n*M*); lower trace: control dialysate of the prefrontal cortex of the rat. Conditions, pump: Shimadzu LC-10ADVP: mobile phase: 75 m*M* sodium acetate, 4 m*M* heptanesulfonic acid, 8% methanol, 0.1 m*M* EDTA, pH 4.7; temperature: 30–32°C; flow-rate: 0.49 ml/min; column: 150×3 mm Hypersil C_{18}, Javelin guard column 20×3 mm; detector: Antec Decade, glassy carbon +550 mV vs. Ag/AgCl (by courtesy of D.E. Johnson and Dr. H. Rollema, Department of Neuroscience, Pfizer Central Research, CT, USA).

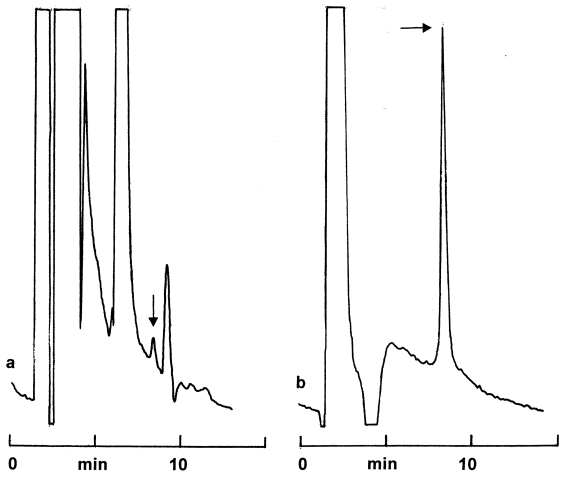

Fig. 4. Analysis of serotonin in the low femtomol range. (a): serotonin (arrow) in a 12-min dialysate of the hippocampus. (b): standard solution, 20 fmol (3.5 pg) serotonin was injected. Conditions: pump: Shimadzu LC-10AD, column: Phenomenex Hypersil C_{18}, 3 μm particles (100×2 mm), mobile phase: 5 g/l ammonium sulphate, 500 mg/l EDTA, 50 mg/l heptane sulphonic acid, 30 μl/l triethylamine and 4.5% methanol (v/v). The pH was 4.65; injection volume: 20 μl; detector: ECD: Intro (ANTEC).

ECD methodology. Further development of mass spectrometry might finally replace the present methods. Fluorescence methods based on capillary electrophoresis (CE) in conjunction with laser-induced fluorescence (LIF) is a well promising alternative. These methods are of great value when short sampling times are used producing samples in the nanoliter range. Recent reports illustrate the high sensitivity of this method: by combining CE and LIF, Renaud and coworkers were able to measure dopamine after precolumn derivatization with naphthalene-2,3-dicarboxaldehyde in 10 s microdialysates samples [61,62].

References

[1] R.D. Johnson, J.B. Justice, Brain Res. Bull. 10 (1983) 567.
[2] U. Ungerstedt, in: C.A. Marsden (Ed.), Measurement of Neurotransmitter Release, Wiley, New York, 1983, p. 81.
[3] B.H.C. Westerink, G. Damsma, H. Rollema, J.B. de Vries, A.S. Horn, Life Sci. 41 (1987) 1763.
[4] H. Benveniste, P.C. Huttemeier, Prog. Neurobiol. 35 (1990) 195.
[5] G. Di Chiara, Trends Pharmacol. Sci. 11 (1990) 116.
[6] A.M.J. Young, Rev. Neurosci. 4 (1994) 373.
[7] B.H.C. Westerink, Beh. Brain Res. 70 (1995) 103.
[8] G. Di Chiara, G. Tanda, E. Carboni, Behav. Pharmacol. 7 (1996) 640.
[9] E.C.M. de Lange, M. Danhof, A.G. de Boer, D.D. Breimer, Brain Res. Rev. 25 (1997) 27.
[10] T.E. Robinson, J.B. Justice) (Eds.), Microdialysis in the Neurosciences, Elsevier, Amsterdam, 1991.
[11] K.M. Kendrick, C. De La Riva, M. Hinton, B.A. Baldwin, Brain Res. Bull. 22 (1989) 541.
[12] R. Tao, S. Hjorth, J. Neurochem. 59 (1992) 1778.
[13] J.J. Rutter, C. Gundlah, S.B. Auerbach, Synapse 20 (1995) 225.
[14] P. Lohnroth, P.A. Jansson, U. Smith, Am. J. Physiol. 253 (1987) E228.
[15] L. Stahle, S. Segersvard, U. Ungerstedt, J. Pharmacol. Meth. 25 (1991) 41.
[16] L.H. Parsons, J.B. Justice Jr., Crit. Rev. Neurobiol. 8 (1994) 189.
[17] H. Yang, J.L. Peters Mic, A.C. Michael, J. Neurochem. 71 (1998) 684.
[18] B.H.C. Westerink, A. Teisman, J.B. de Vries, Naunyn-Schmiedeberg's Arch. Pharmacol. 349 (1994) 230.
[19] M.G.P. Feenstra, M.H.A. Botterblom, J.F.M. van Uum, J. Neurochem. 70 (1998) 1104.
[20] H. Benveniste, J. Drejer, A. Schousboe, N.H. Diemer, J. Neurochem. 49 (1987) 729.
[21] H. Benveniste, N.H. Diemer, Acta Neuropath. 74 (1987) 234.
[22] A. Imperato, G. Di Chiara, J. Neurosci. 4 (1984) 966.
[23] B.H.C. Westerink, J.B. de Vries, J. Neurochem. 51 (1988) 683.
[24] W.J. Drijfhout, R.H.A. Kemper, P. Meerlo, J.M. Koolhaas, C.J. Grol, B.H.C. Westerink, J. Neurosci. Meth. 61 (1995) 191.
[25] B. Fumero, T. Guadalupe, F. Valladares, F. Mora, R.D. O'Neill, M. Mas, J.L. Gonzalez-Mora, J. Neurochem. 63 (1994) 1407.
[26] W.J. Drijfhout, A.G. van der Linde, S.E. Kooi, C.J. Grol, B.H.C. Westerink, J. Neurochem. 66 (1996) 748.
[27] C.D. Blaha, in: H. Rollema, B.H.C. Westerink, W.J. Drijfhout) (Eds.), Proc. 5th Int. Conference in vivo Methods, University Center for Pharmacy, Groningen, 1991, p. 56.
[28] M.C. Walker, P.T. Galley, M.L. Errington, S.D. Shorvon, J.G.R. Jefferys, J. Neurochem. 65 (1995) 725.
[29] A.C. Newton, J.B. Justice Jr., Anal. Chem. 66 (1994) 1468.
[30] A.D. Smith, J.B. Justice Jr., J. Neurosci. Meth. 54 (1994) 75.
[31] B.H.C. Westerink, H.M. Hofsteede, G. Damsma, J.B. de Vries, Naunyn-Schmiedeberg's Arch. Pharamcol. 337 (1988) 373.
[32] M. Santiago, B.H.C. Westerink, Naunyn-Schmiedeberg's Arch. Pharmacol. 342 (1990) 407.
[33] W. Timmerman, B.H.C. Westerink, Synapse 26 (1997) 62.
[34] K. Starke, M. Gothert, H. Kilbinger, Physiol. Rev. 69 (1989) 864.
[35] S.Z. Langer, Pharmacol. Rev. 32 (1981) 337.
[36] P. Blier, C. Bouchard, Br. J. Pharmacol. 113 (1994) 485.

[37] F. Artigas, V. Perez, E. Alvaraez, Arch. Gen. Psychiatry 51 (1994) 248.
[38] B. Moghaddam, B.S. Bunney, J. Neurochem. 54 (1990) 1755.
[39] G.G. Nomikos, M. Iurlo, J.L. Andersson, K. Kimura, T.H. Svensson, Psychopharmacology 115 (1994) 147.
[40] M. Volonté, E. Monferini, M. Cerutti, F. Fodritto, F. Borsini, J. Neurochem. 69 (1997) 182.
[41] G. Tanda, F.E. Pontieri, G. Di Chiara, Science 276 (1997) 2048.
[42] G. Di Chiara, Eur. J. Pharmacol. 375 (1999) 13.
[43] B.H.C. Westerink, H.F. Kwint, J.B. deVries, J. Neurosci. 16 (1996) 2605.
[44] M. Van Gaalen, H. Kawahara, Y. Kawahara, B.H.C. Westerink, Brain Res. 763 (1997) 56.
[45] F.J. Bosker, A. Klompemakers, H.G. Westenberg, Eur. J. Pharmacol. 333 (1997) 147.
[46] M.T. Taber, H.C. Fibiger, Neuroscience 76 (1997) 1105.
[47] B. Schilstrom, H.M. Svensson, T.H. Svensson, G.G. Nomikos, Neuroscience 85 (1998) 1005.
[48] P.T. Kissinger, C. Refshauge, R. Dreiling, R.N. Adams, Anal. Lett. 6 (1973) 465.
[49] J.H. Knox, J. Jurand, J. Chromatogr. 142 (1977) 651.
[50] P.T. Kissinger, Anal. Chem. 49 (1977) 883.
[51] T. Zetterstrom, T. Sharp, C.A. Marsden, U. Ungerstedt, J. Neurochem. 41 (1983) 1769.
[52] E.D. Abercrombie, R.W. Keller Jr., M.J. Zigmond, Neuroscience 27 (1988) 897.
[53] S. Sarre, Y. Michotte, C.A. Marvin, G. Ebinger, J. Chromatogr. 582 (1992) 29.
[54] A.J. Carter, J. Chromatogr. 660 (1994) 158.
[55] N.H. Chen, M.E. Reith, J. Neurochem. 63 (1994) 1701.
[56] L. L'Heureux, T. Dennis, O. Curet, B. Scatton, J. Neurochem. 46 (1986) 1794.
[57] P. Kalen, M. Kokaia, O. Lindvall, A. Bjorklund, Brain Res. 474 (1988) 34.
[58] P. Kalen, R.E. Strecker, E. Rosengren, A. Bjorklund, J. Neurochem. 51 (1988) 1422.
[59] F.A.J. Vanderhoorn, F. Boomsma, A.J. Manintveld, M.A.D.H. Schalekamp, J. Chromatogr. 487 (1989) 17.
[60] M. Yamaguchi, T. Yoshitake, K. Fujino, K. Kawano, J. Kehr, J. Ishida, Anal. Biochem. 270 (1999) 296.
[61] F. Robert, L. Bert, L. Lambas-Senas, L. Donoroy, B. Renaud, J. Neurosci. Methods 70 (1996) 153.
[62] L. Bert, F. Robert, L. Denoroy, B. Renaud, in: H. Rollema, E. Abercrombie, D. Sulzer, J. Zackheim (Eds.), Monitoring Molecules in Neuroscience, State University of New Jersey, Newark, 1999, p. 35.