On-line Monitoring of Biogenic Amines in Microdialysates

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ALEXYS® OMD Analyser for on-line measurement of *in vivo* microdialysates

Introduction

In vivo microdialysis of neurotransmitters has become an invaluable tool to study neurotransmission in brain of living animals. Trough a microdialysis probe, cerebrospinal fluid can be sampled and subsequently analyzed by HPLC with electrochemical detection (ECD).

Direct on-line measurement of the microdialysis sample stream has benefits over analysing fractionated and stored samples at a later moment:

- No sample degradation (no longer sample storage in vials)
- Zero sample loss (no longer fractionation into vials)
- No need of fraction collector and autosampler
- Direct feedback about the course of the experiment

HPLC with ECD has been the method of choice for neurotransmitter analysis, due to its sensitivity. The neurotransmitter metabolites are typically found in subnano- to nano-molar concentrations and are often present in higher concentrations than the neurotransmitter themselves.

The ALEXYS® Analysers were especially designed to measure low neurotransmitter levels in very small samples volumes. Typical detection limits for NA, DA and 5-HT are better than 100 pmol/L (< 0.5 fmol).

In this poster we present **two new Analyzers for on-line measurement of *in vivo* microdialysates**:

- ALEXYS Monoamines: 8 neurotransmitters and metabolites every 12 min (multi component)
- ALEXYS Time Resolution: 2 neurotransmitters every 3 minutes (highest time resolution)

ALEXYS® OMD Monoamines Analyzer

- All monoamines & metabolites
- Analysis of each sample on an optimised dual channel HPLC/ECD system

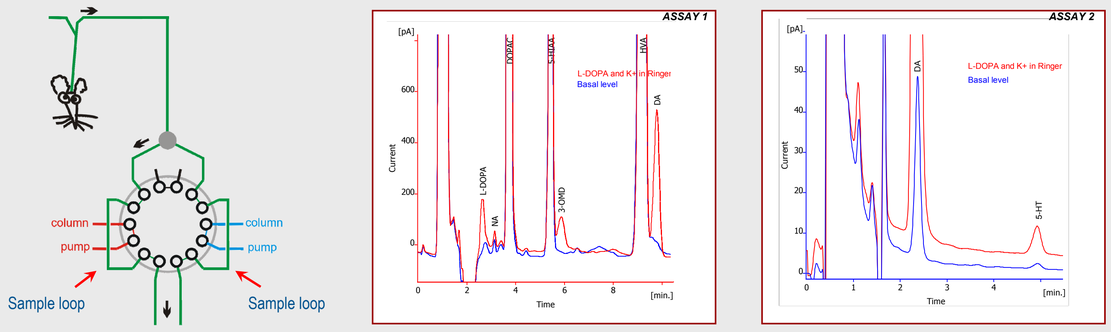

*Figure 1 **Left**: Schematics of on-line microdialysis sampling with the ALEXYS OMD Monoamines analyzer with simultaneous data collection form the two parallel running HPLC/ECD channels. Analysis and loading of subsequent sample occurs simultaneously. **Right**: Chromatograms of rat brain microdialysate. The blue traces show the basal levels and the red traces were recorded after stimulation of the brain with L-DOPA and K+ in the microdialysate solution.*

The growing interest in measuring the response of as many as possible neurochemical components and the need to have data points with short time intervals, is quiet demanding. By on-line analyzing the in-vivo microdialyates on an optimized dual channel HPLC/ECD system these challenge has been overcome. A sample splitter and a 14-port valve with 2 loops connected in parallel is used to collect the microdialysates and inject them in 12 min intervals. The analysis time is used to collect the next sample.

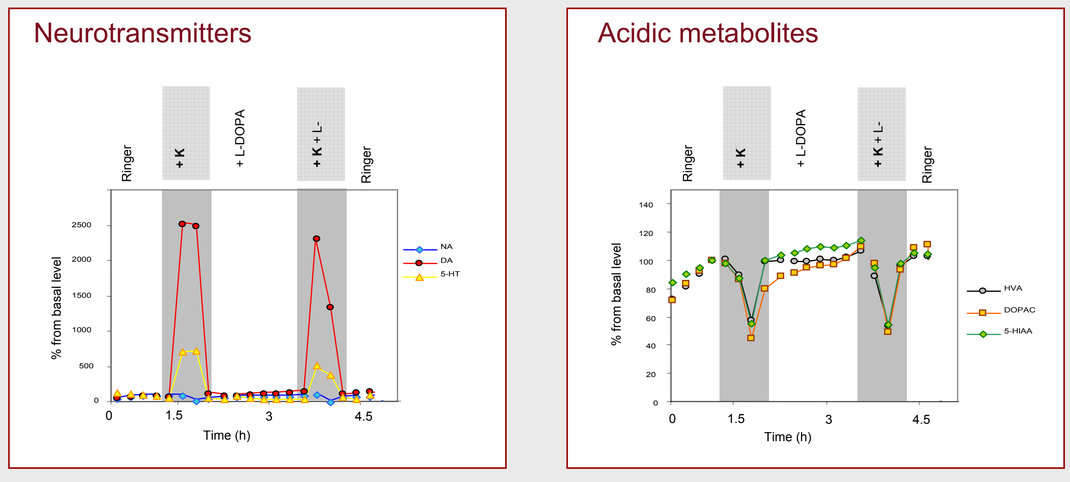

Figure 2: Response plots obtained from in-vivo microdialysis of rat brain and response to different additives in the microdialysate Ringer solution. Data obtained with ALEXYS OMD Monoamines analyzer.

Conclusions

Using the ALEXYS® OMD Monoamine analyzer for the on-line measurement of in vivo microdialysates results in:

- Direct response measurement
- Measurement of all monoamines and metabolites every 12 minutes
- Highest sensitivity for all components (dual channel)
- Savings in test animals, more data from single rodent

ALEXYS® Time Resolution analyzer

- DA/5-HT or NA/DA
- Time resolution ≤ 3 min

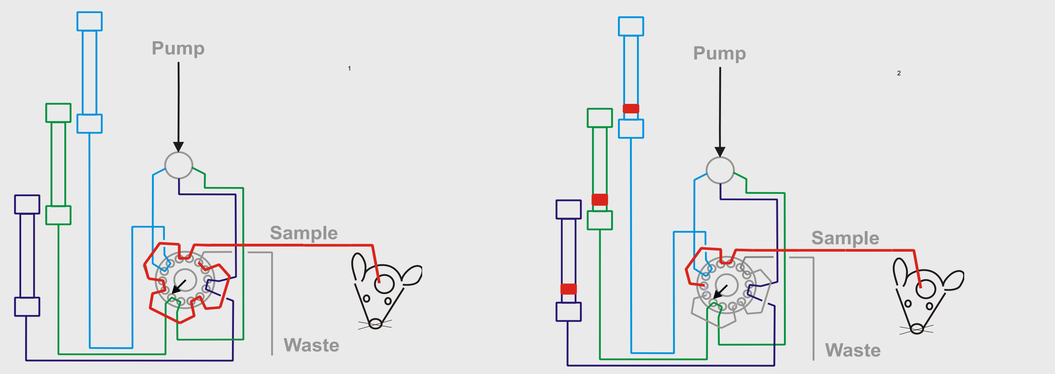

Figure 3: Schematics of on-line microdialysis sampling for improved time resolution. Three parallel 50 x 1 mm HPLC columns are used for fast analysis of neurotransmitters. Analysis and loading of subsequent sample occurs simultaneously.

Conventional sampling of once every 10 or 20 minutes is not sufficient to accurately describe fast neurochemical responses that occur within 30 minutes. To improve time resolution, a 14-port valve with 3 loops connected in series is used to directly collect the microdialysate. The content of these loops is then simultaneously injected on 3 HPLC channels that run the same analysis, and the analysis time is used to collect the following three samples. In this way, time resolution can be improved to 3 minutes.

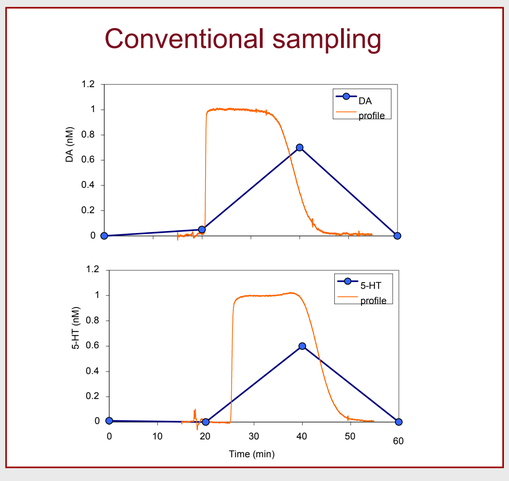

Fig. 4. Conventional sampling, resulting in data collection once every 20 min

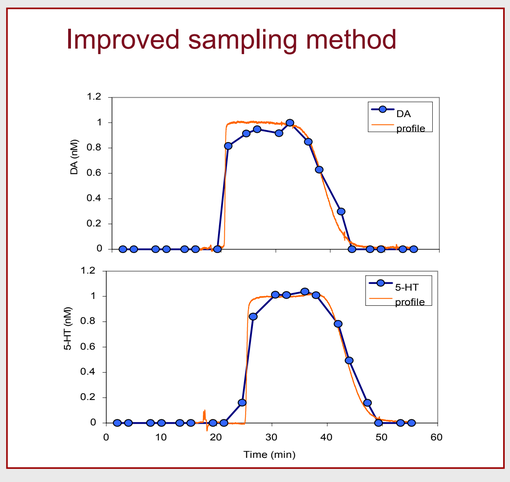

Fig. 5. New sampling method with 3 min time resolution for accurate description of neurochemical response.

Conclusions

Using the ALEXYS® OMD Time Resolution analyzer for the on-line measurement of *in vivo* microdialysates results in:

- Fastest possible time resolution (≤ 3 minutes sampling rate)
- Easy response plot generation (macros provided)
- Savings in test animals, more data per rodent
- No need of fraction collector and autosampler