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Enhanced microdialysis of neuropeptides★✪

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An enhanced microdialysis method for neuropeptides is described and some preliminary results of this novel approach are presented. The enhancement is achieved by adding a vehicle (solid support) to the perfusion fluid in order to increase the diffusion coefficient across the membrane and efficiently transport the analytes towards the detector. The microdialysis samples are desalted and then analyzed on an electrospray ionization orthogonal time-of-flight mass spectrometer. The preliminary results show major increase in signal when comparing this new approach of microdialysis with ordinary microdialysis.

Neurotransmitters are a group of small molecules used by the nervous system to transfer chemical messages between individual cells. The neurotransmitters are stored in axon terminals until the terminal receives an action potential from its respective cell body. The neurotransmitters are then released into the extracellular space and the chemical messages are transferred. Peptides are one major group of neurotransmitters which is responsible for a variety of functions, e.g. mediation of pain (substance P), pain reduction (enkephalins), regulation of water balance (vasopressin) and regulation of blood pressure (neurotensin). The analysis of neuropeptides is challenging due to the fact that the extra-

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Abbreviations: ECF, extracellular fluid; ESI-OTOF MS, electrospray ionization orthogonal time-of-flight mass spectrometry.

cellular environment of the tissue is both physically and chemically complex, the concentration of neuropeptides is usually in the nM–pM range and the neurotransmission processes are often rapid.

Microdialysis, as an *in vivo* sampling technique, is a continuous method that makes it possible to investigate biochemical events in the extracellular fluid (ECF) in animals and human tissues [1]. The principle of microdialysis sampling is to simulate the function of a capillary blood vessel by implanting a probe consisting of a hollow fiber membrane permeable to water and molecules smaller than the cutoff of the membrane. The membrane is then perfused with a liquid solution (perfusate) with the same properties as the ECF considering the ionic composition and pH. The perfusate will try to equilibrate with the ECF by diffusion in both directions and small solutes will be transported across the membrane. The molecular cut off of the membrane is usually 5–30 kDa, so the transport of larger molecules, such as proteins and other macromolecules, will not occur across the membrane [2].

There are at least three different approaches to increase the recovery. First, one can increase the duration of dialysis by reducing the flow rate and increasing the membrane surface area. Second, one can increase the size and quantity of the membrane pores. Finally, one can increase the diffusion efficiency by adding an affinity additive to the perfusate. This will also facilitate the handling of the analyte and reduce non-specific loss.

The aim of this project is to develop a high recovery microdialysis method that is able to collect trace amounts of different neuropeptides in the extracellular space of living tissues. This is done by adding a vehicle (solid support) to the perfusate. Since this is an issue for a patent application, no details of the exact composition of the perfusate can be given in this paper. The dialysate should then be desalted and analyzed on an electrospray ionization orthogonal time-of-flight mass spectrometer (ESI-OTOF MS).

MATERIALS AND METHODS

A microdialysis sampling setup were constructed as illustrated in Fig. 1. The membrane was a polyamide (PA) membrane with a cut-off of 20 kDa. The contact area of the membrane and the sample was 45 mm.

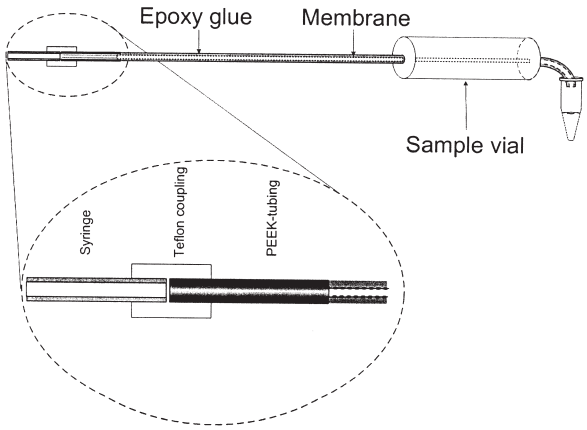

Figure 1. A scheme of the *in vitro* microdialysis setup.

The sample consisted of a 3.3 μM 12-peptide standard including a wide range of peptides regarding hydrophobicity and relative molecular mass (see Table 1). The microdialysis was performed with a stop flow method, where the perfusate was pumped to the sampling area with a high flow rate, halted there for a specified time and then flushed out and collected in a vial. Two perfusates were used, one with just Ringer's solution (artificial ECF) and the other with Ringer's solution with a solid support (patent pending). The time the perfusates were in contact with the sample was either 30 s or 2 min.

After collection the microdialysis sample was desalted and prepared for ESI-OTOF MS (Jaguar, Leco) by elution of the peptides from

Table 1. The peptides in the sample standard

Name	M_r	Sequence
Angiotensin II	1046	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
[Arg8]-Vasopressin	1084	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2 [Disulfide bridge: 1–6]
Bombesin	1620	pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Bradykinin	1060	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Bradykinin fragment [1–5]	554	Arg-Pro-Pro-Gly-Phe
Leucine-enkephalin	556	Tyr-Gly-Gly-Phe-Leu
LH-RH	1182	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
Methionine-enkephalin	574	Tyr-Gly-Gly-Phe-Met
Neurotensin	1673	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
Oxytocin	1007	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2 [Disulfide bridge: 1–6]
Somatostatin	1637	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys [Disulfide bridge: 3–14]
Substance P	1348	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

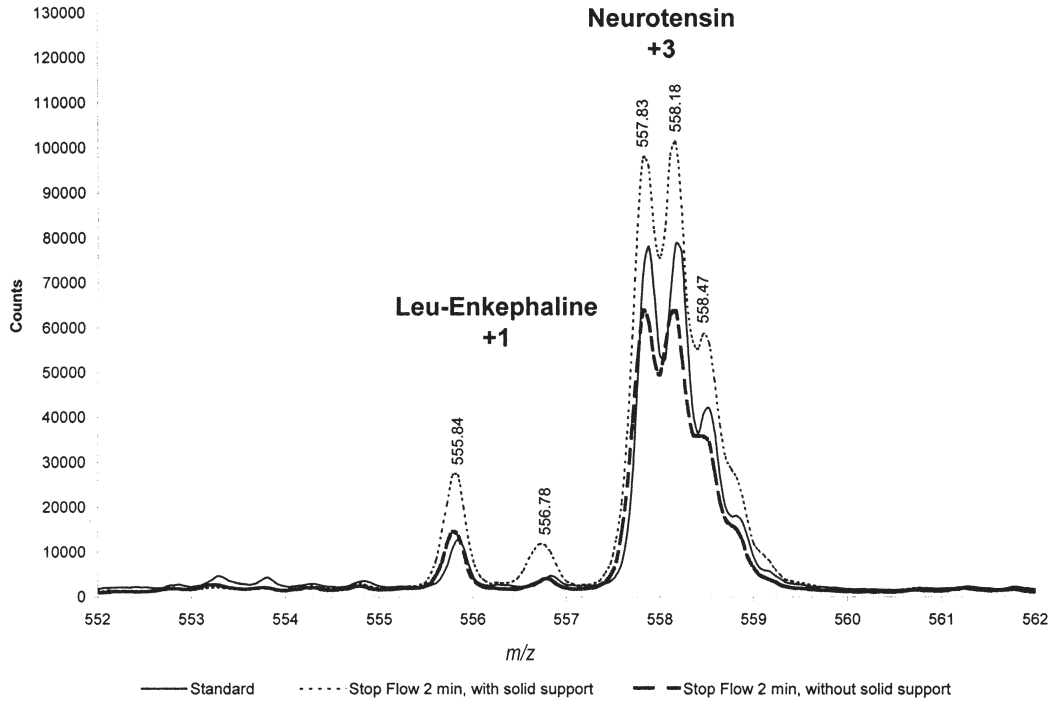

Figure 2. Part of the ESI-OTOF spectra obtained from three samples, standard, stop flow 2 min with solid support and stop flow 2 min without solid support.

the solid support with 50:50 acetonitrile: MilliQ water, with 0.1% formic acid. The flow-rate was set to 500 nL/min and a stable electrospray was obtained at the potential of 2450 V.

RESULTS AND DISCUSSION

The results obtained so far are preliminary and final conclusions can therefor not yet be made. Figure 2 shows part of the spectra ob-

tained from a 2-min stop flow microdialysis with and without solid support. The tendencies from these preliminary data show that the recovery greatly increases when using a solid support in the perfusate, as well as increased stop flow duration. It may also be noted that neurotensin gives the highest intensity while Leu-enkephalin gives almost no signal at all in the mass spectrum. The reason for this needs to be investigated in more detail.

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