Analytical Methods

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Solid-Phase Microextraction: A Complementary In Vivo Sampling Method to Microdialysis**

*Erasmus Cudjoe, Barbara Bojko, Inés de Lannoy, Victor Saldivia, and Janusz Pawliszyn**

Given the intricate organization of the brain, tissue sampling for chemical profiling studies have always been a challenging task. It is often exceptionally difficult to obtain homogeneous samples for in vitro/ex vivo experiments without altering or losing valuable information. The obvious approach has been to develop in vivo analytical methods that may cause minimal perturbation to this complex chemical network so as to improve overall reliability of acquired information. Methods such as biosensors and microdialysis (MD) are among sampling methods applied to in vivo brain chemical profiling studies despite their unique challenges. MD is a well-established in vivo analytical sampling method used over the years for monitoring often low-molecular-weight hydrophilic compounds from the interstitial space. The successful application of the method to neuroscience, especially monitoring of neurotransmitters, led to its expansion to a wider range of analytes, including drugs,[1] metabolites,[2] and peptides.[3] A major challenge, however, associated with MD is its difficulty in sampling hydrophobic compounds. Hydrophobic compounds are often highly protein-bound and bind to the MD probe and tubing, thereby affecting relative recovery. The addition of modifiers, such as bovine serum albumin, glycerol in water,[4] or cyclodextrin,[5] is among the approaches that have been used to prevent hydrophobic interactions and to improve relative recoveries. But these techniques often may complicate the pharmacology of the neurological analytes, as the additives are known to interact with the tissue surrounding the probe.[6] Thus, in typical global metabolomics studies, for example, the composition of a measured metabolome can be significantly affected by the analytical procedure, leaving the analysts with results which likely do not adequately reflect accurate composition of the metabolome during sampling.[7] In effect, it will compromise the already challenging efforts in diagnosis, prognostics, and searching for potential biomarkers for therapeutic purposes. Herein, we demonstrate a novel application of solid-phase microextraction (SPME) for in vivo sampling for brain study. For the first time an application of in vivo SPME as a complementary method to MD for brain-tissue bioanalysis has been presented. Our technique was first validated against MD in targeted analysis of selected neurotransmitters. Their complementary nature was subsequently shown in global profiling of the brain metabolome. From the profiling study, SPME detected groups of lipids such as gangliosides, fatty acids, and lysophospholipids, which are of particular interest in relation to neurodegenerative diseases.

SPME derives its selectivity from the extracting sorbent type. Thus, SPME provides the needed flexibility to analysts to tailor investigations to specific biologically hydrophilic/hydrophobic compounds. For a global study of the metabolome, however, the sorbent choice is one of low selectivity; that is, the sorbent chemical property must enhance simultaneous extraction of hydrophilic and hydrophobic biochemical species. A unique advantage of the new biocompatible in vivo SPME probe is, it prevents extraction of proteins and other bio-interferences owing to the small pore size of the coating and the adhesive biocompatibility, and thus minimizes matrix effect significantly. Furthermore, the in vivo characteristics guarantees enriched chemical information for tissue bioanalysis over other methods when coupled to analytical techniques. Herein, we introduce in vivo SPME and MD coupled to liquid chromatography mass spectrometry (LC-MS) to study the chemical components of the brain extracellular fluid in freely moving rat. Briefly, the approach involved surgically implanting the two probes (SPME and MD), respectively, into the left and right hemispheres of the striata of freely moving rats for continuous chemical monitoring over a period of time. The SPME probe is a simple device placed in a commercial MD guide cannula without extended tubes to an external pumping system contrary to what is typical for the MD probe. Samples collected for both MD and SPME were subjected to a reversed reverse-phase chromatographic separation on a pentafluorophenyl stationary phase in a positive-mode mass spectrometry analysis.

Knowing that SPME sorbent can extract relatively wider range of analytes, including hydrophobic biomolecules contrary to MD, initial investigations focused on the effectiveness of the sampling technique for monitoring small polar endogenous compounds in a targeted metabolomics study. Subsequently, we simultaneously monitored the effect of single dose ($10\,mg\,kg^{-1}$) fluoxetine on multiple neurotransmitters: dopamine (DA), serotonin (5-HT), gamma aminobutyric acid (GABA), and glutamic acid (GA) using both SPME and MD. The purpose was to evaluate the ability of SPME to monitor at each time point changes in multiple neurochemicals with a single probe similar to MD. The

[*] E. Cudjoe, Dr. B. Bojko, Prof. J. Pawliszyn
Department of Chemistry, University of Waterloo
200 University Avenue, Waterloo, N2L 3G1 (Canada)
E-mail: janusz@uwaterloo.ca
Homepage: http://spme.uwaterloo.ca
Dr. I. de Lannoy, V. Saldivia
InterVivo Solutions
120 Carlton St., Toronto, ON (Canada)

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selected neurotransmitters represented compounds with a wide range of pK_a values (acidic to basic properties). This is critical, as the chemical information obtained at the time of sampling is dependent on the sorbent type. Furthermore, through this investigation we are able to demonstrate that an in vivo SPME method, like MD, is capable of capturing changes in the concentration of endogenous biomolecules during sampling. After the sampling processes, all SPME-derived samples were analyzed by LC coupled to tandem MS (Thermo Scientific TSQ Vantage), whereas MD derived samples were analyzed at NoAb BioDiscoveries using LC coupled to electrochemical detection. Figure 1 shows the ability of in vivo SPME sorbent to capture changes in the concentration of serotonin after a single dose intraperitoneal (i.p.) injection of fluoxetine at time zero.

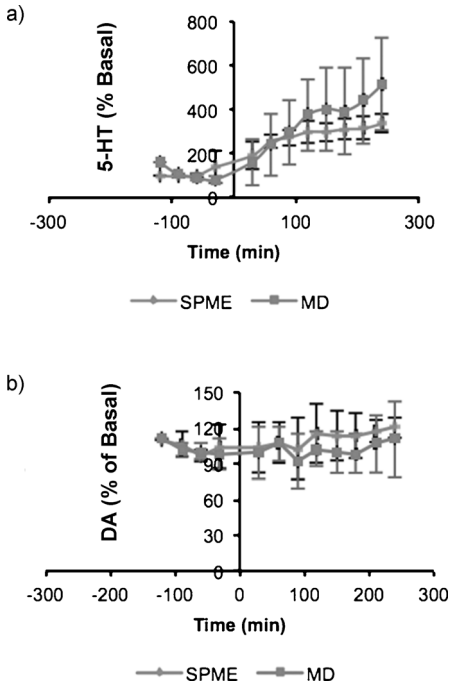

Figure 1. Results for in vivo solid-phase microextraction (SPME) and microdialysis (MD) study of the effect of single-dose fluoxetine on selected neurotransmitters. Changes in serotonin (a) and dopamine (b) concentrations (relative to the mean basal concentration) in the rat brain extracellular fluid determined by SPME and MD methods following an intraperitoneal (i.p.) injection of fluoxetine (10 mg kg^{-1}) at time $t = 0$. The results are expressed as the mean ± standard error for 12 rats.

The analyses from the neurotransmitters demonstrated that in vivo SPME is capable of monitoring basal concentrations of multiple neurotransmitters (GABA, GLU, DA, and 5-HT) with the insertion of a single probe at each time point (Supporting Information, Figure S3). However, monitoring by MD resulted in sample analyses of fewer analytes (DA and 5-HT) with a single implanted probe, owing to the limited sample volume (that is, perfusate flow rate) required for enhanced relative recovery. Despite the differences in detection techniques, both methods captured changes in 5-HT concentration relative to the basal concentration of the striatum following a dose of fluoxetine. The SPME data showed the added advantage of better precision and repeatability compared to the MD technique data. Although temporal resolution is a challenge, this can be improved with relatively shorter coating exposure time in the brain. Sensitivity consequently may be compromised in certain cases owing to the lesser amount of analyte extracted; however, the combination of SPME with very sensitive MS makes it a viable alternative worth exploring. The relatively large error bars observed with MD may be due to susceptibility of the analytical technique to matrix interference, which is often minimal or negligible in certain bioanalytical applications with SPME. Generally, both methods did not exhibit changes in the concentration of dopamine after i.p. injection of fluoxetine (Figure 1 b). Owing to the fact that SPME requires the insertion of multiple probes, the potential changes in neurotransmitter level and/or brain tissue damage induced by probe insertion was assessed. This was carried out by injecting dosing vehicle and monitored basal concentration of neurotransmitters and also performed histology study (Supporting Information, Figure S1). Limits of detection (LOD; three times signal-to-noise ratio) for all analytes ranged from 0.009 to 0.024 ng mL^{-1}, whereas limit of quantitation was computed based on ten times the signal-to-noise ratio (0.030 to 0.08 ng mL^{-1}) with GABA having the highest LOQ value (0.08 ng mL^{-1}). Details of the SPME calibration method and precision of both methods for in vivo extraction of 5-HT and DA are presented in the Supporting Information.

Subsequently, we extended the approach to global chemical profiling of the striata of freely moving rat. After the in vivo sampling, the dialysates and SPME samples were separated by LC and the analytes were detected using the orbitrap mass spectrometer in positive-ionization mode. The MD data, as anticipated, was biased toward polar/hydrophilic compounds with lower LogP values. The SPME data, on the contrary, was biased to hydrophobic/less polar compounds (Supporting Information, Tables S1 and S2). Among the metabolites detected by SPME are compounds from groups (carnitines, gangliosides, fatty acids, and lysophospholipids, including lysophosphatidic acid and lysophosphatidylethanolamine) of particular interest to clinicians because of their involvement in various neurological diseases and disorders. To date, no effective in vivo analytical method that is applicable to lipids analysis is available. Current methods are mainly based on brain tissue collection, homogenization, and extraction of lipids with organic solvents. The disadvantage here is that unstable compounds can be lost with long and laborious multistep in vitro methods, whereas in vivo analysis rather enriches knowledge about physiological and pathological metabolic pathways by extracting short-lived species. Consequently, the results of the former will not fully reflect the real metabolome profile and some information about metabolic pathways can be missed or misinterpreted. For example, in vivo SPME was shown elsewhere to capture elusive species and eliminated the need for quenching step required for in vitro conventional methods.[7] Another remarkable information from the data was that MD captured hydrophilic dipeptides and aliphatic amino acids, which were

absent in the SPME extract (Supporting Information, Table S2). This establishes the complementary nature of the two methods, especially for global metabolomics studies, by improving the overall metabolite coverage. That said, it is worth mentioning that with SPME methods, by choosing the appropriate extraction phases, such as ionic liquids and HILIC, hydrophilic compounds (amino acids, including aliphatic species) can be successfully determined (data not published). It should also be noted that dilution factor used in the current metabolomics studies influenced overall coverage of metabolites by SPME and MD, respectively. Nonetheless, it can be postulated that on a single diagnostic platform, in vivo SPME could be used successfully as a complementary method with MD to provide a full spectrum of the compounds extracted and possibly provide better information about the neuroactivity of the brain. This would enhance the chance for the discovery of biomarkers related to various neurological disorders and diseases.

With the introduction of the new biocompatible SPME probes, we have laid the foundation for various potential clinical applications. For example, cutaneous monitoring and sampling of tumors and other tissues in humans for disease biomarkers can be exploited. Another potential clinical application is investigating neurological disorders through in vivo SPME sampling of cerebrospinal fluid. In this case, higher temporal resolution may be attained with in vivo SPME sampling owing to faster mass transfer in fluids. The potential benefits of μSPME probes (for example, performing intracellular sampling), with submicrometer/nanometer particle coatings, still remains unexploited. Another interesting application would be to demonstrate in vivo SPME capability to monitor different regions of the brain(for example cortex and striatum) simultaneously using a single probe. The approach will in principle show the effectiveness of space-resolved in vivo SPME for monitoring chemical or drug concentration gradients in a specific region of tissue. Consequently, it will easier to ascertain the level of drug toxicity in specific brain regions. Such a preliminary study on the distribution of carbamazepine in the cortex and striatum of freely moving rats following drug administration was performed simultaneously with a single SPME probe. The results showed no statistical difference in concentration of carbamazepine in these two regions (data not published). However, space-resolved SPME was demonstrated elsewhere in studying the distribution of chemicals in plants[8] and bio-concentration of pharmaceuticals in different portions of the fish.[9]

Experimental Section

The biocompatible mixed-mode probes (C18 with benzenesulfonic acid, 45 μm thickness and 4 mm long, Supelco Inc.) were preconditioned overnight prior to use in a methanol/water (1:1 v/v) mixture for in vivo SPME experiments. Commercial 4 mm MD probes were obtained from CMA Microdialysis. Probes were inserted into opposing brain hemispheres at the same time for basal concentrations of neurotransmitters in a targeted analysis. Subsequently, probes were used to capture changes in the concentrations of neurotransmitters after 10 $mg\,kg^{-1}$ fluoxetine dose. Along with the targeted analysis of neurotransmitters, a non-targeted study was carried to obtain global chemical information in both SPME extracts and dialysates. For the SPME method, immediately after sampling/extraction, the probes were briefly rinsed in purified water and subsequently desorbed using acetonitrile/water (2:3 v/v) with 0.1% formic acid. The extracts were analyzed directly with the reverse-phase LC-MS/MS method on the TSQ Vantage (targeted analysis) and the Exactive Orbitrap instruments (non-targeted analysis) using a pentafluorophenyl stationary phase. Full information on in vivo sampling and LC-MS methods are given in the Supporting Information.

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