Optimization of analytical approach based on SPME-LC-MS/MS for the analysis of selected endocannabinoids in brain regions

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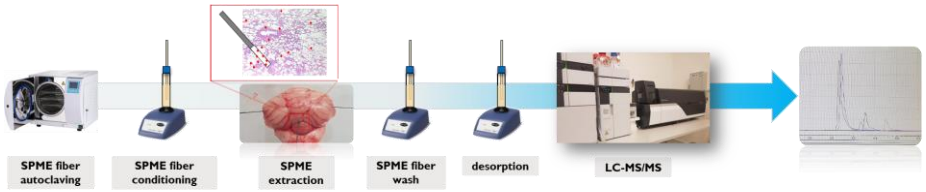
INTRODUCTION

The endogenous cannabinoid system is involved in several physiological processes, including energy metabolism and memory processes (1). This system consists of different types of receptors (e.g., **CB1** and **CB2**), ligands and enzymes involved in the metabolism of endocannabinoids (**ECBs**) (2). The purpose of this study was to optimize the SPME-LC-MS/MS methodology for the analysis of four selected endocannabinoids, namely anandamide (**AEA**), 2-arachidonoyl glycerol (**2-AG**), N-arachidonoyl dopamine (**NADA**), 2-arachidonyl glyceryl ether (**2-AGe**) in selected brain regions. In addition, the level of receptors of endocannabinoid system, namely CB1 and CB2 in brain samples was estimated, and correlated with the level of ECBs.

SPME-LC-MS/MS ANALYTICAL WORKFLOW



METHODOLOGY

- SPME biocompatible probes with C18 coating (extraction phase) were applied for the sampling of 3 brain regions (cerebellum, cerebral cortex and striatum) of young (1 month old) and old (24 months old) female and male rats (Fig. 1).
- C18 probes were introduced into selected parts of brain for 30 min static extraction, and then obtained extracts were subjected to instrumental analysis. LC-MS/MS analysis were performed with the use of Kinetex XB-C18 column (100×2.1mm, 2.6 µm). Mobile phase A was composed of water with 0.1% FA, mobile phase B was composed of ACN with 0.1%FA. Total analysis time was 10 min (Table 1).
- 3 brain tissue sections (40 µm each) were stained with the use of primary antibodies against CB1 receptors (policlonal rabbit antibody, 1:200 concentration) and GFAP receptors (monoclonal mouse antibody, 1:300 concentration); secondary antibodies were CY3 goat antirabbit (1:600) and Alexa488 goat antimouse (1:150), respectively.

Table 1. Details of MS conditions.

analyte	Precursor (m/z)	Product (m/z)	CE	Q1	Q3	tr (min)
2-AG	379.2	287.2	-15.00	-12.00	-20.00	4.3
		269.0	-18.00	-12.00	-13.00	
2-AGe	365.2	273.1	-12.00	-11.00	-29.00	4.8
		121.0	-20.00	-11.00	-20.00	
AEA	348.2	62.1	-20.00	-10.00	-25.00	3.7
		287.2	-13.00	-10.00	-20.00	
NADA	440.2	137.0	-27.00	-11.00	-25.00	4.2
		153.0	-18.00	-11.00	-10.00	
		119.1	-40.00	-12.00	-24.00	
AEA- d11 (IS)	359.4	62.1	-27.00	-11.00	-25.00	3.7
		298.2	-18.00	-11.00	-10.00	
		214.1	-12.00	-12.00	-24.00	

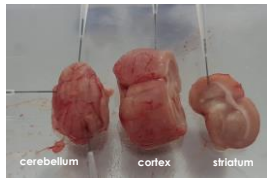


Fig.1. SPME extraction of ECBs with the use of 4 mm C18 fibers. In each experiment, 30 min static extractions were performed. Three brain regiones, namely cerebellum, cortex and striatum were sampled with the use of 3 fibers, 1 fiber and 1 fiber, respectively. For each group (based on age and sex) 3 rat's brain were used.

RESULTS

- SPME conditions for the analysis of endocannabinoids in brain samples were optimized, and 30 min extraction time and 60 min desorption time. A mixture of MetOH/IPA (50:50, v/v) was selected for the desorption of endocannabinoids into silinized glass vials (Fig.2 and Fig.3)

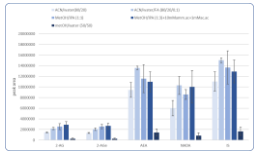


Fig.2. The extraction efficiency of analyzed ECBs and IS (at a concentration of 50 ng/mL) from PBS solution with the use of SPME and different desorption mixtures.

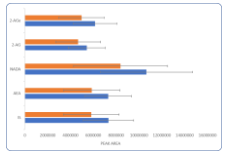


Fig.3. Comparison of the performance of regular glas vials vs. silanized glass vialse during the extraction of analyzed ECBs and IS (at a concentration of 50 ng/mL) from PBS solution with the use of SPME.

- 2-AG and AEA were extracted by SPME fibers from all analyzed brain regions, however, the level of 2-AG was significantly higher in comparison to the level of AEA in each brain region analyzed, which confirms previous observations (Fig.4).
- The level of AEA was relatively constant in cerebellum and striatum during the development of male and female rats; whereas significant differences in the level of AEA were observed in the cortex
- NADA and 2-AGe were not detected in each brain section analyzed, suggesting either lability of those compounds or their fast biotransofrmation in the brain.

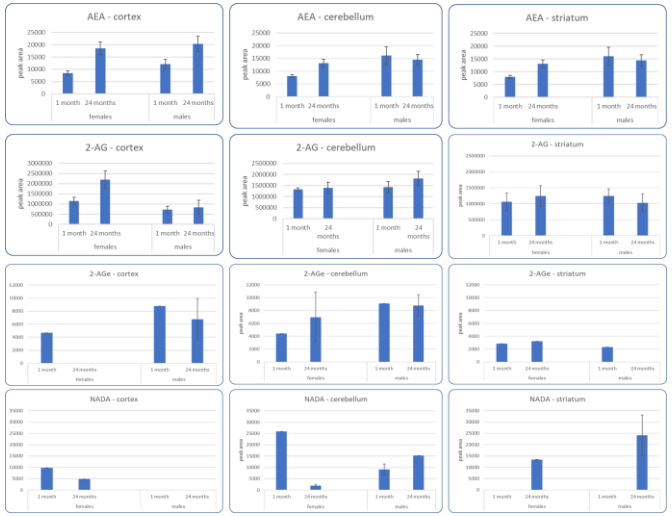


Fig.4. Comparison of the level of AEA, 2-AG, NADA and 2-AGe in cerebellum, cortex and striatum regions. The extraction of analyzed ECBs was performed with the use of SPME fibers. After desorption, deuterated IS (AEA-d11) at the concentration of 50 ng/mL was added into each desorption mixture, and next samples were subjected to LC-MS/MS analysis.

- immunohistochemical staining of three brain tissue sections revealed differences in the distribution of CB1 receptors in female and male rats at different stages of development (Fig.5)
- CB1 receptors predominate in cortex, however, more receptors were observed in old rats in comparison to young ones
- no differences in the distribution of CB1 receptors were detected in striatum of female and male rats

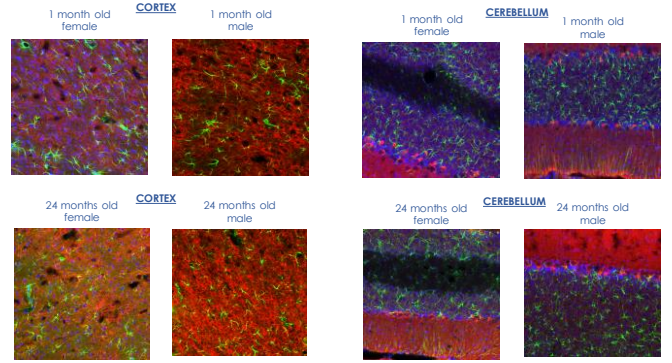


Fig.5. Immunohistochemical staining of CB1 receptors in cortex and cerebellum.

CONCLUSIONS

- High-throughput technique based on SPME along with LC-MS/MS method was developed for the analysis of trace level of selected ECBs in brain tissue samples
- Optimized SPME-LC-MS/MS method was applied for the extraction of four ECBs (AEA, 2-AG, NADA, 2-AGe) from intact (non-homogenized) brain regions (cerebellum, striatum, cortex)
- Singnificant differences in the level of particular ECBs were observed in young and old rats, and also between female and male rats at different stages of development.
- The level of ECBs correlated well with the number of CB1 receptors detected in the analyzed brain sections; increasing number of those receptors during the development of rats was observed in cortex and cerebellum.

REFERENCES

(1) S. Zou and U. Kumar, Cannabinoid receptors and the endocannabinoid system: Signaling and function in the central nervous system, *Int. J. Mol. Sci*. 19 (3) 2018
(2) N. Ueda, K. Tsuboi, and T. Uyama, Metabolism of endocannabinoids and related N-acylethanolamines: Canonical and alternative pathways, *FEBS J.* 280 (9) 2013