Neurochemistry International 57 (2010) 655–661

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

# Effects of post-mortem intervals on regional brain protein profiles in rats using SELDI-TOF-MS analysis

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ARTICLE INFO

*Article history:*
Received 29 March 2010
Received in revised form 23 July 2010
Accepted 3 August 2010
Available online 11 August 2010

*Keywords:*
Brain region vulnerability
Post-mortem interval
Protein stability
Tissue apposition
Tissue proteomics

ABSTRACT

Identification of disease-associated proteins is critical for elucidating CNS disease mechanisms and elaborating novel treatment strategies. It requires post-mortem tissue analysis which can be significantly affected by the collection process, post-mortem intervals (PMIs), and storage conditions. To assess the effect of time and storage conditions on brain protein stability, SELDI-TOF-MS protein profiles were assessed in rat frontal cortex, caudate-putamen, hippocampus and medulla samples collected after various PMIs (0, 6, 12, 24, 48, and 72 h) at 4 °C or at room temperature (RT) storage. Regions of interest were isolated from cryosections (tissue apposition, TA), or micropunched from cryosections apposed on filter paper (paper apposition, PA), and applied onto an NP20 ProteinChip® array. Protein alterations, while greater at RT than at 4 °C, were detected at 6 h then differentially evolved in the various brain regions, with greater alterations in the caudate-putamen (60%) and the cortex (48%). Overall, our sensitive analytical method allowed unveiling of different patterns of protein susceptibility to PMI and to storage temperature in the various brain regions. Some protein peaks were altered in all brain regions and may potentially serve as markers of the PMI status of the brain, or for reference values when studying new proteins. Changes in disease-related proteins within post-mortem samples can be greatly affected by PMI and storage conditions, particularly when studying fragile and/or low abundant protein/peptides in tissues sampled from the caudate-putamen and neocortex.



## 1. Introduction

Brain neurochemistry provides an invaluable contribution to pathophysiological studies of diseases. Although experimental models allow specific hypotheses to be tested, human material is irreplaceable. The quality of human material is affected by a variety of factors including patient recruitment, stringency of diagnostic criteria, post-mortem intervals (PMIs) and storage conditions, particularly temperature. In our human tissue dataset, the range of PMIs is generally from 6 to 72 h (Machaalani and Waters, 2008). Given the variability in collection method, site, and storage conditions of human post-mortem samples, it is important to understand how these conditions affect protein stability.

Exploration of brain neurochemistry includes the study of neurotransmitters, genes, RNA and more recently proteins. Proteins and RNA have fragile structures compared to DNA, but proteins provide the most information, since changes in RNA do not always translate into changes in protein expression (Machaalani and Waters, 2003). Since proteins are the main effectors of physiological and pathological processes, proteomic methodologies are increasingly used to identify potential biomarkers of brain disease. Analytical approaches in proteomics include 2D-PAGE protein separation and liquid chromatography combined with mass spectrometry (MS) techniques (LC/TOF-MS, LC/MS–MS), and MALDI-TOF (Fountoulakis and Kossida, 2006). To date, proteomic methods have rarely been used to identify the effects of PMI-related changes in brain proteins (Fountoulakis et al., 2001; Voshol

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doi:10.1016/j.neuint.2010.08.002

et al., 2003; Crecelius et al., 2008; Hunsucker et al., 2008). Previous studies have applied the 2D-PAGE/MALDI-TOF method to analyze whole brain tissue homogenates (Fountoulakis et al., 2001; Voshol et al., 2003; Hunsucker et al., 2008) and single brain regions (Crecelius et al., 2008; Ferrer et al., 2007). Because susceptibility to injury greatly varies in different brain regions, more region-specific analysis is required to properly detail relevant PMI-related changes.

The method of surface-enhanced laser desorption ionization-time of flight-mass spectrometry (SELDI-TOF-MS) allows easy screening of tissue proteins using ProteinChip® arrays. The latter provide various surface chemistries that enable capture and analysis of specific subclasses of proteins from tissue and other biological samples. The technique has been recently applied to neuroproteomics (Geng et al., 2006; Mei et al., 2006; Novikova et al., 2006). Using the SELDI technique, we developed two new brain tissue preparations; either direct apposition of the cryosectioned tissue onto the ProteinChip® array (tissue apposition; TA) (Bouamrani et al., 2006), or first apposing the cryosection onto a filter paper (paper apposition; PA) to facilitate micropunching of minute regions of interest (Machaalani et al., 2007). We found that these two easy-to-use tissue collection techniques significantly enriched the protein profile and increased peak amplitude, therefore improving the discriminatory power of SELDI proteomics (Bouamrani et al., 2006; Machaalani et al., 2007).

The relevance and vulnerability of particular brain regions to diseases vary, as do their structural anatomy, chemistry and their susceptibility to degradation according to PMI and storage temperatures. In the current study, we selected the caudate-putamen, a structure that we have previously studied (Machaalani et al., 2007), the neocortex and hippocampus because of their increased vulnerability to anoxia-ischemia (Cervos-Navarro and Diemer, 1991), and the medulla, because of its role in the control of cardiorespiratory, sleep and arousal regulation (Coote, 1982). The latter is particularly relevant to the pathophysiology of sleep apnea and the sudden infant death syndrome (SIDS), as areas of interest to our laboratory.

The aim of our study was to combine the advantages of our tissue collection methods with SELDI-TOF-MS analysis to compare the effects of different PMIs and storage temperatures on several rat brain regions that are likely to present differential vulnerability to post-mortem protein alterations.

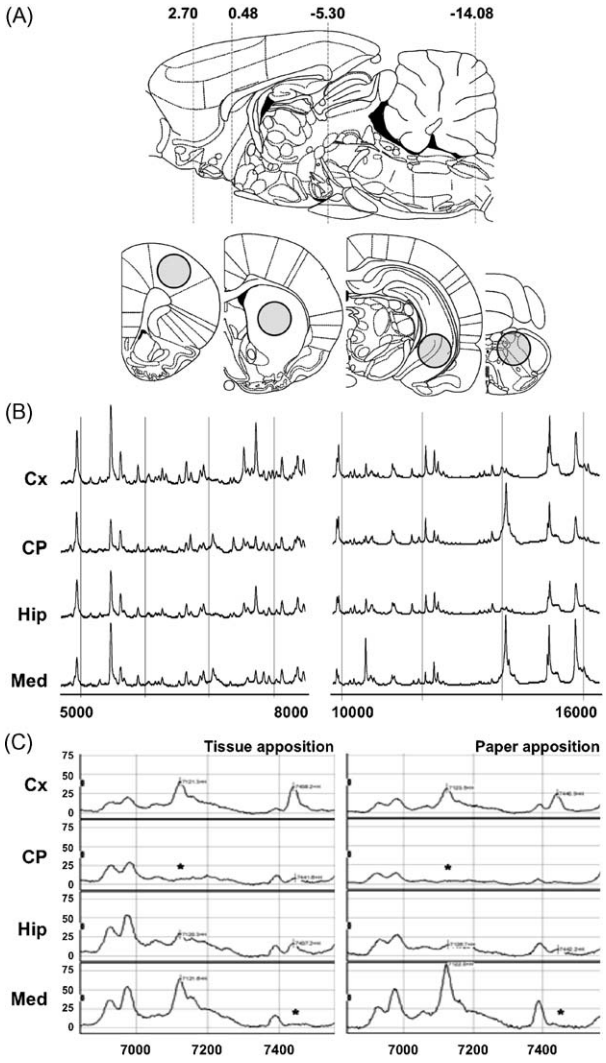


**Fig. 1.** (A) Micropunched brain areas for SELDI analysis and (B) corresponding peak profiles obtained by applying tissue apposition with (C) enlargement within the range 6800–7600 showing examples of peaks with the two apposition methods. *Example of peaks differing amongst the four brain regions, with $m/z$ 7121 peak missing in the caudate-putamen and $m/z$ 7436 peak missing in the medulla. Note the good homology between the tissue and paper apposition methods in detecting these differences. Abbreviations: Cx, cortex; CP, caudate-putamen; Hip, hippocampus; Med, medulla.

## 2. Methods

### 2.1. Brain tissue collection and storage

A total of 24 adult male Wistar rats (250 g, Janvier, France) were studied. The rats were sacrificed using $CO_2$ and brains were collected and frozen immediately (T0), or after varying PMIs at 4 °C for 6, 12, 24, 48, and 72 h, or at room temperature (RT; 23–24 °C) for 6 and 12 h ($n$ = 3 rats per group). Time points and storage temperatures were chosen to mimic PMI conditions encountered with SIDS. At the end of their respective PMI, the rats were decapitated, the brain extracted, separated in two hemi-brains along the midline and further cut into three blocks, at the superior colliculus separating the brainstem from the rest of the brain, and through the thalamus. Blocks were snap frozen in liquid nitrogen, and stored at −80 °C. Experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe, European Treaties ETS 123, Strasbourg, 18 March 1986).

### 2.2. Sample preparation

Four brain regions were studied (Fig. 1). Serial coronal sections of these regions were collected from the left hemi-brain for the frontal neocortex, the caudate-putamen, the hippocampus, and the medulla, at AP 2.70, 0.48, −5.30, −14.08 mm from Bregma, respectively (Paxinos and Watson, 1998). Two methods of sample preparation were assessed including tissue apposition (TA) and paper apposition (PA) as previously described (Bouamrani et al., 2006; Machaalani et al., 2007). Briefly for the TA method, 4 $mm^2$ of the region of interest was isolated from 10 μm cryosections and directly apposed onto the ProteinChip® spot. After a short incubation period, matrix was applied before reading (see below). The PA method was similar to TA method, but included an additional step of apposition of 20 μm cryosections onto a filter paper (903 ProteinSaver® paper, Whatman) which simplifies cutting out the region of interest. Samples were dried for 2 h at RT, then 2 mm micropunches were taken from the four regions of interest using a Harris Micro-Puncher (Whatman). The micropunch was applied (non-tissue-side) onto adhesive tape and briefly (60 s) rehydrated with 1 μl of distilled water on its tissue-side, then apposed onto the ProteinChip® spot (see Supplemental Figure). Notwithstanding the drying step, the paper method gives similar protein profiles when compared to tissue apposition, suggesting limited protein degradation due to the paper method (Machaalani et al., 2007).

### 2.3. ProteinChip® array preparation and SELDI analysis

We selected the ProteinChip® NP20 array (Ciphergen, Bio-Rad Laboratories) which mimics normal-phase chromatography with silicate functionality, as we did not want to assess subclasses of proteins but global protein alterations; 2 × 0.8 μl of a saturated solution of sinapinic acid in 50% acetonitrile and 0.5% trifluoroacetic acid was added onto ProteinChip®-apposed samples. The arrays were read with the ProteinChip® Reader PCS4000 model (Ciphergen, Bio-Rad Laboratories), at laser energy of 6000 nJ, and data averaging 530 laser shots per spot and per spectrum were analyzed with ProteinChip® software 3.2.1 (Ciphergen, Bio-Rad Laboratories). The instrument and spectra were calibrated using the All-in-one protein standard (Ciphergen, Bio-Rad Laboratories) and the following peaks were selected