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Use of surface enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS) to study protein expression in a rat model of cocaine withdrawal

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Abstract

Surface enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS) is an analytical technology for proteomic analysis that combines chromatography and mass spectrometry. At present, this technology is most commonly being exploited for the simultaneous measurement of numerous proteins in serum, but has also been utilized in organ tissue, although rarely in the brain. We applied SELDI-TOF MS technology to study protein expression in the brain of rats withdrawn from repeated cocaine exposure. Our goals were to optimize sample preparation and ProteinChip® Array protocols for brain tissue, to verify the reproducibility of SELDI-TOF mass spectra and to determine whether SELDI-TOF MS detects differentially expressed proteins in cocaine- versus saline-treated rats. Consequently, we have developed an optimal protocol and generated a reproducible spectral pattern with six dominant peaks in all test samples. We have detected two smaller peaks (*m/z*: 5179, 5030) that were significantly increased ($p < 0.05$) in cocaine-treated rats compared to saline-treated rats. In summary, the application of SELDI-TOF MS to the study of protein expression in a rat model of cocaine withdrawal is feasible and has the potential to generate new hypotheses.

Keywords: Brain; Cocaine; Proteomics; Mass spectrometry; SELDI; SELDI-TOF

1. Introduction

The study of biological processes has been traditionally driven by hypothesis-based methods, limiting the focus of investigations to the involvement of only one or a few protein targets in a specific biological function. Current discovery approaches in proteomics have enabled researchers to analyze hundreds of proteins (and/or their peptide fragments) simultaneously and rapidly (Morris and Wilson, 2004). However, while the simultaneous study of numerous proteins in a cell, tissue or organism (Fountoulakis, 2004) is of great interest and has been applied to a large variety of studies, many of these new methodologies are still under development and need to be continually validated as the tools are applied to novel systems or to answer novel questions. In contrast to clinical studies, where the eventual goal is diagnostic, discovery methods such as these can be useful in basic research as hypothesis generating mechanisms.

One organ system for which the development of proteomic analyzes is in its infancy is the nervous system, as few proteomic analyzes have been conducted in this system. The ability to observe changes in the expression of a multitude of proteins simultaneously offers vast opportunities for neuroscientists hoping to unveil new proteins or currently unknown relationships between proteins. However, the complexity of the brain, which occurs at several levels including the multitude of brain areas, various cell types (neurons, glia, endothelial cells) within each area (Morrison et al., 2002), an assortment of neuronal cell subtypes, as well as complex subcellular architecture (Williams et

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al., 2004), presents a sundry of obstacles that must be overcome when conducting such studies. Thus, methodological validation must be conducted prior to initiation of the proteomic analyzes.

Proteomic analyzes are comprised of two steps: separation of a protein mixture and identification of the separated proteins by various analytical methods, mainly mass spectrometry (Aebersold and Mann, 2003; Fountoulakis, 2000; Griffin and Aebersold, 2001). Classical protein separation methods, such as two-dimensional gel electrophoresis (2DGE), are labor-intensive, often require large amounts of sample (~100 μg protein) and are generally limited to the study of proteins that are neither strongly ionic nor hydrophobic (Williams et al., 2004). Furthermore, 2DGE has a poor ability to separate proteins of low molecular weight, especially those less than 10 kDa (Williams et al., 2004). Therefore, these classical proteomic methods are sub-optimal for the separation of most membrane proteins and limit the range of proteins available for analysis. However, recent advances in protein separation technologies have overcome some of these limitations.

Surface enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS) technology is a combination of conventional chromatography and mass spectrometry (Ciphergen Biosystems, Fremont, CA) and consists of three major processes: protein separation on the surface of a ProteinChip® Array, TOF-MS detection of the ionized protein and peptide molecules and data processing. Unlike 2DGE, which separates proteins based on isoelectric point (pI) and molecular weight, proteins are separated in SELDI-TOF MS according to the chromatographic properties of the proteins (Hutchens and Yip, 1993). The surface of each ProteinChip® Array is designed to capture a subset of proteins from the sample based on chromatographic interactions between the chip surface and the protein or peptide molecules (Merchant and Weinberger, 2000). There are a variety of chip types available including cation exchange, anion exchange, hydrophobic and metal affinity exchange that are derivatized with classic chromatographic separation moieties (Merchant and Weinberger, 2000). The availability of multiple types of chip surfaces allows analysis of a broader range of proteins. For example, many membrane-bound proteins are hydrophobic and difficult to detect by 2DGE. The hydrophobic "H50 chip" adsorbs hydrophobic proteins through reverse phase or hydrophobic interactions (Ciphergen Catalog, 2005a) enabling better detection of these types of proteins (Yuan and Carmichael, 2004).

SELDI-TOF MS technology has several features attractive for the study of proteomics in neural disease processes. Low sample volumes (0.5–300 μl) and sample amount (as few as 2000 cells, or 1–2 μg of protein) can be employed (Wittke et al., 2004), which may be critical for examining proteins within a specific brain region, as the size of most brain areas are small and thus only provide modest sample volumes. In addition, the chip surfaces allow a better retention of low mass proteins (<20 kDa) than 2DGE (Merchant and Weinberger, 2000) and the sensitivity of this technology allows detection of individual peaks containing as few as 50 fmol of protein or peptide molecules (Vorderwulbecke et al., 2005), which is imperative for detecting low abundance proteins that are likely present in a dynamic environment, such as the brain. It is important to note that although protein and peptide molecules are analyzed by MS after the on-chip separation, the mass spectral data from SELDI-TOF MS predict only the molecular weights of the protein or peptide fragments and provide a platform for comparison of relative protein expression levels among samples (Merchant and Weinberger, 2000). As with 2DGE, subsequent protein identification and hypothesis testing (especially when small numbers of samples are used in experimental situations) must be performed using additional methods, such as matrix assisted laser desorption/ionization (MALDI)-TOF MS.

We are interested in identifying coordinate protein expression patterns that occur in the brain during withdrawal from repeated exposure to the psychostimulant cocaine (De La Garza and Cunningham, 2000; Filip et al., 2004; Loftis and Janowsky, 2000; Perrotti et al., 2005). SELDI-TOF MS technology, through rapid screening of multiple proteins, provides a tool for new hypothesis generation and novel protein discovery in such mechanistic studies of cocaine withdrawal. However, since SELDI-TOF MS technology has not yet been applied to the study of protein expression in brain tissue (Williams et al., 2004), we first conducted a series of studies to establish the parameters necessary to apply these tools in experimental neuroscience.

We began by optimizing sample extraction and ProteinChip® Array preparation protocols for brain tissue and verifying the reproducibility of SELDI-TOF MS spectra. In order to generate reliable and reproducible protein profiles during optimization, we used pooled samples (see Section 2.4) to develop a consistent set of conditions for obtaining protein extracts, for applying them to the ProteinChip® Arrays and for generating spectra. We quantified the variation of both *m*/*z* and peak intensity by testing these pooled samples on different instruments, different chips and different days. Once the protocols were optimized, we measured SELDI-TOF spectra of protein extracts from each individual cocaine- or saline-treated rat to determine whether differences were detectable between the two groups.

In this experiment, rats were injected with cocaine (15 mg/kg) twice a day for 7 days. This treatment protocol has been shown previously to result in behavioral alterations in rats during cocaine withdrawal, inducing a progressive increase in cocaine-induced hyperactivity that persisted following cessation of the treatment (Kalivas and Duffy, 1993; Kozell and Meshul, 2004; Lau et al., 1991). In the present experiments, rat brain tissue was collected 3 days after the last treatment injection and subjected to SELDI-TOF MS analyzes in order to detect protein expression changes associated with documented behavioral alterations seen during this span of time (Loftis and Janowsky, 2000; Perrotti et al., 2005).

The ventral tegmental area (VTA) was chosen for SELDI-TOF analysis because of its importance in mediating the behavioral alterations seen during withdrawal from chronic exposure to cocaine (Kalivas and McFarland, 2003; Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000; White and Kalivas, 1998). Changes in expression levels of individual proteins in the VTA during cocaine withdrawal have been identified (Beitner-Johnson and Nestler, 1991; Beitner-Johnson et al., 1992; Hope et al., 2005; Vanderschuren and Kalivas, 2000). For instance,