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The Role of Blue Native/ SDS PAGE in Depression Research

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1. Introduction

Depression is common in medical patients and is associated with diminished health status and increased health care utilization (DiMatteo et al., 2000). It is characterized by low mood, low self-esteem, inappropriate guilt, thoughts of death and suicide, diminished concentration, loss of interest or pleasure in normally enjoyable activities, and disturbance of sleep and appetite. Approximately 3% of depressed patients in the United States commit suicide and around 60% of people who commit suicide have major depressive disorder (MDD) or another mood disorder (NIH). Over the past decade, evidence has accumulated to suggest that patients with depression were significantly impaired on tasks of attention, executive function, memory and psychomotor speed. These findings suggest deficits in cognitive function, attention, visual and verbal memory and learning, as vulnerability marker for major depressive disorder (Channon & Green, 1999; Gohier et al., 2009; Weingartner et al., 1981).

Synapse is the key structure for various brain functions such as learning and memory. The synapse consists of a pre-synaptic bouton and a post-synaptic spine separated by a synaptic cleft, and is connected via diverse types of cell adhesion molecules located in the opposing membranes of the pre- and post synapse. The fundamental role of the synapse in neurotransmission and plasticity is mediated by various kinds of neurotransmitter receptors and signal transduction proteins(Kaindl et al., 2008). Proteins responsible for the mechanisms of neurotransmitter release have been investigated in detail (Lang & Jahn, 2008; Murthy & De Camilli, 2003). The expression and modification of synaptic proteins is dynamically regulated, depending on the neuronal activity. This process is called synaptic plasticity, and is thought to be the cellular basis of learning and memory (Bagal et al., 2005; Maffei, 2011; Martin & Morris, 2002). Plasticity-induced changes can occur on both sides of the synapse by altering Ca^{2+}-dependent neurotransmitter release from the presynaptic nerve terminal and/or modifying the response to neurotransmitter in the postsynaptic neuron(Evans & Morgan, 2003). Abnormalities of synapse physiology have been implicated in a number of brain disorders, for example, mental retardation, drug addiction,

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neurodegenerative diseases and so on(Battaglia, 2011; Grant et al., 2009; Koob et al., 1998). Cognitive impairment suggests changes in the expression of numerous synaptic proteins in depressive disorder.

Although recent studies have identified several candidate genes and proteins associated with major depression, the roles of the resultant proteins remain unknown. The techniques of proteomics provide us with powerful tools to find the differentially expressed proteins in various conditions. Two-dimensional electrophoresis based on isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second dimension is the most commonly used method for protein separation and is a powerful proteomic technique to display differentially expressed proteins and posttranslational modifications in proteomic approaches. 2-DE can separate thousands of proteins based on their differences in charge and size(Gorg et al., 2000). However, isoelectric focusing is still not the method of choice for separation of hydrophobic membrane proteins, which represent at least 30% of all proteins coded in the genome, despite recent developments and systematic evaluation of detergents(Luche et al., 2003; Santoni et al., 2000). Several solutions have been developed to overcome the technical difficulties represented by the analysis of membrane proteins. For example, fractionation of cell lysates and biochemical enrichment has been reported to reduce pattern complexity on 2D gels and thus to improve visualization of low abundance proteins (Zhang et al., 2010). Alternatively isoelectric focusing can be eliminated (one-dimensional electrophoresis), modified, or judiciously replaced with a different separation technique such as blue native (BN)-PAGE. This technique allows the characterization of many proteins that cannot be resolved using conventional IEF SDS-PAGE(Wittig et al., 2006). Today, hundreds of proteins are identified by BN-PAGE which has had an enormous impact on the investigation of the respiratory chains and photosynthetic complexes(Schagger & von Jagow, 1991). When combined with SDS-PAGE, BN-PAGE allows an assignment of proteins to their protein complexes and display highly hydrophobic proteins in two dimensions. Recently, BN/SDS PAGE is used for the one-step isolation of microgram amounts of membrane protein complexes from different types of samples. Some publications demonstrate that BN-PAGE is also a valuable technique for the identification of physiological protein-protein interactions. The application of BN-PAGE will expand to the analysis of further receptors in the plasma membrane and in the membranes of cellular organelles, to the analysis of protein-chaperone interactions, and to the detection and analysis of antigen antibody interactions. This technology is beginning to be applied to clinical diagnostics of human mitochondrial disorders; to determine native masses and oligomeric states; to identify physiological protein–protein interactions and for studies of neurotransmitter assembly(Eubel et al., 2005; Nijtmans et al., 2002). This alternative 2D method was successfully used to screen for differentially expressed proteins and protein complexes in cystic fibrosis knock-out mice and salt-induced halotolerant alga, indicating that this approach is applicable to obtain a comprehensive map of the differentially expressed synaptic proteins occurring in the depression(Brouillard et al., 2005; Katz et al., 2007). We will discuss the capabilities of BN-PAGE with a focus on its potential use in depression research.

2. The potential role of blue native/SDS PAGE in depression research

The development of depression as well as recovery from depression is most likely accompanied by a change in protein or protein complexes expression profiles in synapse.

Mitochondria provide most of the energy for brain cells by the process of oxidative phosphorylation. Mitochondrial abnormalities and deficiencies in oxidative phosphorylation have been reported in individuals with schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD) in proteomic studies(Lindholm et al., 1997; Rollins et al., 2009). Although significant progress has been made in our understanding of the depression, the molecular mechanisms underlying this disorder are still not clear. Novel technology such as blue native/SDS PAGE may offer new insights into this devastating illness because this technique not only allows the separation of protein sets for analysis but also provides information on their native interactions. Here, we will show the potential role of blue native/SDS PAGE as a tool to elucidate the differentially expressed proteins and protein complexes in synaptic plasma membrane and mitochondrial during depression and explore what could be done with this proteomic method to offer an insight to the pathogenesis of depression, biomarker establishment and drug development.

2.1 The working principle of BN-PAGE

Blue native polyacryamide gel electrophoresis (BN-PAGE) enables analysis of native (nondissociated) protein–protein interactions, particularly those involving hydrophobic membrane proteins. It was initially developed by Schagger and von Jagow to separate intact and functional mitochondrial membrane protein complexes responsible for oxidative phosphorylation(Schagger & von Jagow, 1991). It is used for the one-step isolation of microgram amounts of membrane protein complexes between 10 and 10 000 kDa from biological membranes and total cell or tissue homogenates. In principle, any nonionic detergent or mild anionic detergent, such as cholic acid derivatives, can be used for the solubilization of biological membranes for blue native PAGE, as long as the detergent can solubilize the desired protein and keep it in the native state. Mild neutral detergents are used for solubilisation of biological membranes, nonionic detergents such as digitonin, Triton X-100, dodecylmaltoside and so on are used for solubilisation the sample. To prevent re-aggregation of hydrophobic membrane proteins, the solubilization buffer contains a high concentration of aminocaproic acid; being a zwitterion, free aminocaproic acid does not migrate in the gel, thus avoiding issues of depletion from the proteins during electrophoresis. Following solubilization of biological membranes and centrifugation, the anionic dye Coomassie blue G-250 is added to the supernatant. G-250 can bind to membrane proteins because of its hydrophobic properties. This binding of a large number of negatively charged dye molecules to protein has several useful effects. (1) It shifts the isolectric point of the proteins to more negative values. As a result all proteins, even basic ones, migrate to the anode irrespective of their original isoelectric points upon electrophoresis at pH 7.5. (2) The excess negative charges on the surfaces of individual dye-associated proteins repel each other. The separation principle relies on binding of Coomassie blue G250 which provides negative charges to the surface of the protein. During migration to the anode, protein complexes are separated according to molecular mass and/or size and high resolution is obtained by the decreasing pore size of a polyacrylamide gradient gel. Thus a mixture of protein complexes of different native charges can be loaded at the top of a native gel, and be resolved according to differences in apparent native molecular weight. Native proteins and complexes migrate as blue bands through BN gels (Devreese et al., 2002; Lasserre et al., 2006; Schamel, 2008; Sunderhaus et al., 2007; Wang et al., 2007).

Protein complex assemblies that are retained during 1D BN-PAGE can be dissociated into the individual protein by applying an orthogonal SDS PAGE for the second dimension, thereby identifying the interaction partners and their stoichiometric ratio. For a second dimension, a lane of the BN-gel can be cut out and incubated in a buffer containing 1% SDS, 1% mercaptoethanol and then placed horizontally on the second dimension gel. This step ensures complete denaturation of the protein complexes necessary for the subsequent separation of their subunits. Loosely stuck in the pores of the gel, the subunits of the protein complexes remain at their position until they are forced electrophoretically into the second dimension gel. Due to the SDS used in the denaturation step (and residual Coomassie) the polypeptides are uniformly negatively charged and are separated according to their molecular weight in the gel. Subunits of a protein complex form a vertical row on the second dimension gel.

2.1.1 Detergents, stock solutions, and buffers

20% dodecyl-b-D-maltoside (wt/vol) (Fluka), dissolved in water; store 1-ml aliquots at –20 °C.

20% Triton X-100 (wt/vol) (Fluka), dissolved in water; store 1-ml aliquots at –20°C.

20% digitonin (wt/vol) (cat no. 37006, 450% purity, used without recrystallization) (Fluka), dissolved in water; store 0.1–1 ml aliquots at –20 °C.

49.5%T, 3%C Acrylamide: 24 g acrylamide, 0.75 g bisacrylamide / 50 ml H_2O

3 x Gel buffer: 150 mM BisTris-HCl, 1.5 M 6-aminohexanoic acid, pH 7.0, adjust pH to 7.0 with HCl at 4°C, store at 4°C.

50% (w/v) Glycerol, store at 4°C.

1 M imidazole/HCl, pH 7.0; store at 7 °C.

10 x Cathode buffer: 0.5 M Tricine, 75 mM imidazole, no need to adjust pH, store at 4°C.

5 x Anode buffer: 0.125 M imidazole, pH 7.0, adjust pH to 7.0 with HCl at 4°C, store at 4°C.

1 M 6-aminohexanoic acid, pH 7.0, adjust pH to 7.0 with HCl at 4°C, store at 4°C.

5% (wt/vol) stock of Coomassie blue G-250 (Serva) (suspend in 500 mM 6-aminohexanoic acid and store at 7 °C).

2.1.2 Prepare the sample: Isolation of synaptic plasma membrane from rat hippocampus

The rats were killed by decapitation, and the hippocampus were dissected quickly and stored on ice. The synaptic plasma membrane (SPM) fraction was isolated essentially as described. First, the hippocampus from 10 rats were combined and homogenized in solution A (0.32 M sucrose, 1 mM $NaHCO_3$, 1 mM $MgCl_2$, and 0.5 mM $CaCl_2$ containing protease inhibitor cocktail) at 4 °C using a hand-held disperser (IKA products, T8 ULTRA-TURRAX, Germany). A low speed (1400g for 10 min) pellet was obtained from the resultant homogenates and washed by resuspending the pellet in the solution A and then homogenized again. All the centrifugation in this experiment was carried out at 4 °C. The second centrifugation was performed at 710g for 15 min. The supernatants were pooled and

centrifuged at 13 800g for 20 min. The resulting pellet was suspended in solution B (0.32 M sucrose and 1 mM NaHCO₃ containing protease inhibitor cocktail). The sucrose gradients, composed of suspended material, 0.85, 1.0, and 1.2 M sucrose were then centrifuged for 2 h at 82 500g. The bands between 1.0 and 1.2 M sucrose were collected and then diluted with solution B. The synaptosome was spun down at 32 800g for 20 min. The synaptosomal pellet was washed and resuspended in 12 mL of hypotonic solution containing 12 mM Tris-HCl (pH 8.1) and osmotic shock was carried out on ice for 45 min under gentle stirring. The resulting SPM was obtained after centrifugation at 20 000g for 30 min.

2.1.3 Blue native-PAGE and SDS-PAGE

BN-PAGE was performed according to the published protocols with minor modifications(Wittig et al., 2006). Briefly, a 4-13% gradient separation gel with a 3.5% stacking gel was poured and stored at 4 °C until further use.Composition of a sample gel and acrylamide gradient separation gel was shown in Table 1. The cathode buffer (7.5 mM Imidazole, 50 mM Tricine)containing 0.02% (w/v) Coomassie Brilliant Blue G250 and the anode buffer (25 mM Imidazole, pH 7.0) were chilled to 4 °C before use.

For BN-PAGE, 3 μL of 50% Glycerol and 3 μL of 5% G-250 were added to 30 μL of sample, which was then loaded onto the sample well. Electrophoresis was begun at 100 V at 4°C. After about 1 h, the cathode buffer was replaced by the same buffer containing 0.002% of G250, and the electrophoresis was continued with voltage at 200 V at 4 °C. The BN gel was fixed and stained with Coomassie Brilliant Blue G250. One typical gel separation was shown in Figure 1. For further separation in a second-dimensional SDS-PAGE, the excised lanes from BN-PAGE were denatured in SDS loading buffer for 2 h. The lanes were then
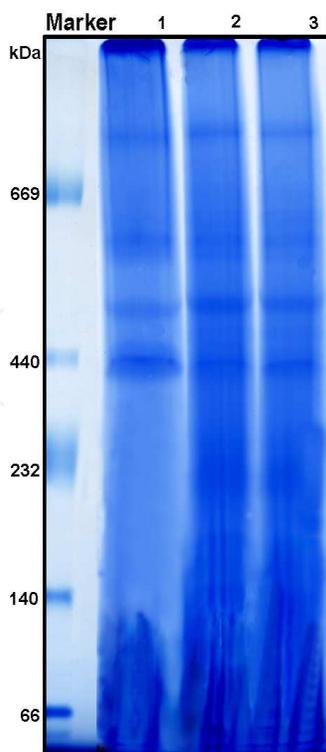

Fig. 1. The Blue Native PAGE of the synaptic plasma membrane fractions.
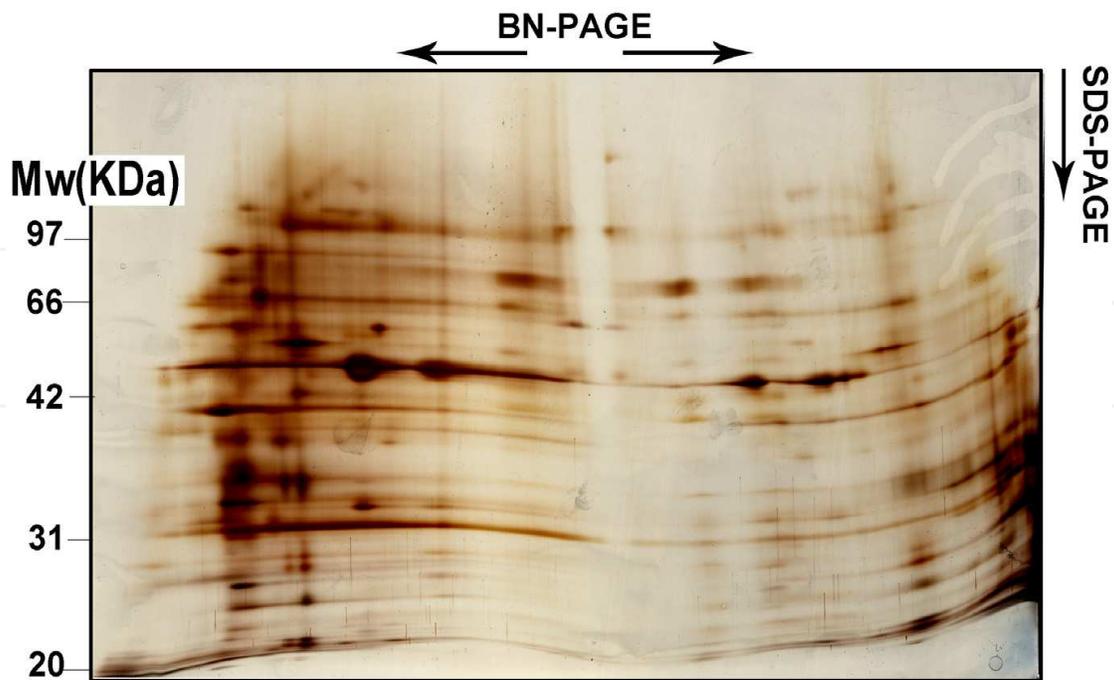
Fig. 2. The SDS-PAGE of the multiprotein complexes bands from Blue Native PAGE.

rinsed briefly with 20 mM Tris buffer. SDS-PAGE was performed using 7.5-12.5% acrylamide gradient gels. The excised lanes were then placed into the SDS-PAGE and sealed with hot agarose solution. Electrophoresis was performed at 20 mA until the front passed into the separation gel and then continued at 45 mA. Proteins were visualized using standard silver staining protocol. One typical gel separation was shown in Figure 2.

	Sample gel	Gradient separation gel	
	3.5% acrylamide	4% acrylamide	13% acrylamide
AB-3 mix	0.44 ml	1.5 ml	3.9 ml
Gel buffer $3\times$	2 ml	6 ml	5ml
Glycerol	-	-	3 g
Water	3.4 ml	10.4 ml	3 ml
Total volume	6 ml	18 ml	15 ml
10% APS	50 µl	100 µl	75 µl
TEMED	5 µl	10 µl	7.5 µl

Table 1. Composition of a sample gel and 4% and 13% acrylamide mixtures to prepare an acrylamide gradient gel.

2.2 Current biological applications of BN/SDS PAGE studies of mitochondrial and synaptic plasma membranes

2.2.1 Current biological applications of BN/SDS PAGE studies of mitochondrial

Mitochondria provide the main energy source for eukaryotic cells, oxidizing sugars and fats to generate ATP via oxidative phosphorylation (OXPHOS). This is accomplished by the

respiratory chain, a series of multimeric enzyme complexes of the inner mitochondrial membrane comprising complex I (NADH-ubiquinone oxidoreductase,EC 1.6.5.3), complex II (succinate-ubiquinone oxidoreductase, EC 1.3.5.1), complex III (ubiquinol-ferricytochrome c oxidoreductase, EC 1.10.2.2), complex IV(cytochrome c oxidoreductase, EC 1.9.3.1), and complex V (F1F0 ATPase). Blue Native polyacrylamide gel electrophoresis (BN – PAGE), followed by immunodecoration, has proved to be invaluable for the analysis of individual respiratory complexes. Individual respiratory complexes also exist in larger supercomplexes, with the largest form (observed at 1700 kDa on BN – PAGE) estimated to contain four copies of complex IV, two copies of complex III, and one copy of complex I (Schagger & Pfeiffer, 2000). Two recent studies identified that complex I stability is reliant on an intact complex III, and this may relate to the association of these complexes within a supercomplex. In addition, destabilized supercomplexes resulting from cardiolipin remodeling defects were detected by BN – PAGE in patients with Barth syndrome, highlighting the importance of supercomplex stability for respiratory chain function (McKenzie et al., 2007; Schagger et al., 2004).

In humans, the respiratory complexes of the mitochondrial inner membrane contain approximately 90 different subunits, with 13 encoded by mtDNA. Assembly of these complexes requires subunit expression from both mitochondrial and nuclear genomes as well as the involvement of various assembly factors, chaperones, and protein translocation components required for targeting and folding of subunits at the inner membrane(Burger et al., 2003).

BN – PAGE has been used to examine the assembly of complex IV, identifying two assembly intermediates that contain mtDNA-encoded subunits. The efficiency of assembly of human respiratory complexes was monitored using radiolabeled, mitochondrially encoded subunits in conjunction with BN PAGE. Intermediate complexes containing newly synthesized mitochondrial DNA-encoded subunits could be observed for complexes I, III, and IV and human cytochrome b was detected as a monomer and as a component of a novel approximately 120 kDa intermediate complex at early chase times before being totally assembled into mature complex III. Furthermore, the researchers showed that BN PAGE was highly suited for the rapid detection of respiratory complex assembly defects in fibroblasts from patients with mitochondrial disease and, thus, has potential diagnostic applications(McKenzie et al., 2007; Wallace, 2005).

The structural organization of enzymes in biological membranes can be very complex, such as ATP synthase, which is usually isolated in monomeric form. However, it appears that ATP synthases are organized as more or less extended functional homooligomeric structures in the inner mitochondrial membrane. Blue native PAGE has been used to isolate ATP synthase as dimers or oligomers. This protein was first isolated as a dimer from yeast mitochondria, using TritonX-100 at very low detergent/protein ratio (Arnold et al., 1998). Dimeric and even oligomeric ATP synthases were later isolated using digitonin from yeast mitochondria. ATP synthases in the mass range 1.5–6 MDa have been isolated from mammalian mitochondria .BN PAGE was also used to detect the oligomycin-sensitive ATPase activity for monomeric and oligomeric ATP synthase and found that oligomerization/monomerization dynamics are not directly involved in regulating ATP synthase activity(Krause et al., 2005).

2.2.2 Current biological applications of BN/SDS PAGE studies of synaptic plasma membrane

Synaptic transmission involves an intricate network of synaptic proteins that forms the molecular machinery underlying transmitter release, activation of transmitter receptors, and signal transduction cascades. It is generally believed that neuronal activity-dependent change of synaptic efficacy is at the basis of learning and memory and is encoded by sequential molecular events at the synapse. Many neuropsychiatric and neurodegenerative diseases, such as Alzheimer's, are thought to involve altered expression of multiple structural and/or metabolic genes and proteins, and therefore are well-suited for proteomic analysis(Kim et al., 2004).

The study of other conditions, such as addiction and mood disorders that likely are secondary to altered expression of proteins involved in neurotransmission or neuroplasticity, can also take advantage of the power of global and narrow protein profiling that proteomics offers, for example, to examine the role of synaptic proteins in different disease states(K. W. Li & Smit, 2007). To date, most proteomic analyses of the brain either considered proteins located within different parts of the cell, or considered differentially expressed proteins in response to development or disease. Those studies lack information about the interaction pattern of these proteins, a very important aspect of the functions that the proteins perform. BN-PAGE offers great promise for the purification of intact protein complexes.

In our lab, BN /SDS-PAGE combined with tandem mass spectrometry, was used to screen multiprotein complexes in synaptic plasma membranes from rat hippocampus. 514 unique proteins were identified, of which 36% were integral membrane proteins. Several multiprotein complexes involved in membrane trafficking and synaptic vesicle exocytosis were identified, such as SNAREs. SNAREs play a role in the docking and fusion of synaptic vesicles to the active zone. The cores of SNARE complexes are composed of syntaxin, synaptobrevin, and SNAP-25. Besides SNAREs, we also found several known SNARE-associated proteins, such as the calcium channel alpha-2 delta-3 subunit, isoform 1 of the syntaxin-binding protein 1, and synaptotagmin-1(X. W. Li et al., 2009).

Most excitatory synapses in the brain use glutamate as a neurotransmitter. Fast signaling at these synapses occurs primarily by activation of amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type and N-methyl-D-aspartate (NMDA)-type glutamate receptors. AMPAR channels comprise heterotetramers of subunits GluR1-4, and were reported to interact with cytoskeletal protein, BiP, AP-2 and NSF (Fukata et al., 2005; Vandenberghe et al., 2005). Three membranes containing AMPA-type glutamate receptor were identified from our analysis.

A potentially novel protein complex, involving syntaxin, synapsin I and Na$^+$/K$^+$ ATPase alpha-1, was further confirmed by co-immunoprecipitation and immunofluorescence staining. We found BN /SDS-PAGE was a powerful tool for the separation of hydrophobic membrane proteins and for the systematically identify synaptic plasma membrane protein complexes. To the best of our knowledge, this was the first synaptic plasma membrane proteome, and the largest data set determined by the BN PAGE strategy. It contributes promising starting points for further studies.

2.3 Future opportunities for BN in depression research

Brain is a high energy demand tissue and therefore more susceptible to reduction of aerobic metabolism. Even slight reduction in brain metabolism will impair judgment, memory and other higher brain function within seconds. Mitochondria provide most of the energy for brain cells by the process of oxidative phosphorylation. Mitochondrial abnormalities and deficiencies in oxidative phosphorylation have been reported in individuals with schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD) in transcriptomic, proteomic, and metabolomic studies. The relationship between mitochondrial dysfunction and unipolar depression has been explored in several studies(Liu et al., 2011). Adult BALB/c mice were exposed to unpredictable chronic mild stress (UCMS) for different periods and differential 2D gel electrophoresis (DIGE) approach was employed to the brain tissue to find the differentially expressed proteins. DIGE analysis revealed marked alterations in the expression of proteins involved in energy metabolism, cytoskeleton, and response to cellular stress. Changes in expression of the protein, such as mitochondrial complex I, II, III, IV, V and enzymes relating to glycolysis were detected. Alterations of translational products linked to mitochondrial function, decreased gene expression for 6 of 13 mtDNA encoded transcripts, reduced of respiratory chain enzyme ratios and ATP production rates, and an increased prevalence of small mtDNA deletions (but not of the common 5 kb mtDNA deletion),were found in the depression in different areas of the brain. Impairment of complex I was seen in prefrontal cortex in all patients with bipolar disorder (Andreazza et al., 2010), and abnormalities of mitochondrial structure in the prefrontal cortex, fibroblasts and lymphocytes in another recent study (Cataldo et al., 2010).

Identifying the differentially expressed mitochondrial proteins is available in a rapidly growing subset of mitochondrial disease patients, yet this is still a minority. No systematic strategies for elucidating the mitochondrial proteome in depression patient or animal model have yet been reported. BN PAGE has been proven generally successful for the identification of integral membrane proteins and protein complexes from mitochondria and can maintain the enzyme activity of the complexes, so it will be powerful in research for identifying biomarker from mitochondrial in depression disorders.

Proteomic studies of hippocampus from a chronic stress rat model of MDD identified 27 differentially expressed proteins which participated in neurogenesis and oxidative metabolism. The authors claimed that cellular plasticity is a key issue for the understanding of the molecular mechanism of MDD (Mu et al., 2007). Protein is the actual effector of biological functions and, therefore, proteomic analysis is a powerful way to deepen our knowledge of the molecular mechanisms leading to depression or promoting recovery. Proteome analyses of HAB/LAB mice model led to the identification of glyoxalase-I as a protein marker downregulated in multiple brain areas and an alternate enolase phosphatase isoform in HAB mice (Mu et al., 2007).

Synaptic transmission involves fusion of neurotransmitter-containing vesicles with the plasma membrane and activation of postsynaptic receptors. In nerve terminals, the arrival of the depolarizing wave triggers the opening of presynaptic calcium channels, producing the calcium influx that induces the fusion of docked synaptic vesicles at the active zones and

elicit release of glutamate-containing vesicles, which activate postsynaptic receptors that can be generally described as having two broad functions. The receptors that mediate the postsynaptic depolarizations that are responsible for initiating the action potential are Na^+-permeable receptors known as AMPA receptors and those that activate signalling and plasticity mechanisms are NMDA receptors and mGluRs (metabotropic glutamate receptors)(Becherer & Rettig, 2006; Schoch & Gundelfinger, 2006; Sheng & Kim, 2002). Synaptosomes are isolated nerve endings of brain tissue and is greatly enriched in proteins involved in synaptic plasticity. Thus synaptosomes are an ideal tool to analyze the global expression of proteins at synaptic sites where many biological targets of antidepressants are located. A few proteomic studies have been carried out on characterization of the synaptic proteome in animal models of depression. A proteomic analysis of synaptosomes obtained from hippocampus (HPC) and prefrontal/frontal cortex (P/FC) of LH rats or their controls, nonlearned helpless (NLH) rats. 2-DE was employed to identify proteins whose expression is differentially regulated in LH vs. NLH rats at synaptic level. Only 2 synaptic proteins (synapsin and synuclein) were identified in the research. The majority of the identified proteins is mitochondrial and signal proteins (Mu et al., 2007). In our study, we found that blue native PAGE was a powerful tool for the separation of synaptic plasma membrane proteins. So we can combine BN/SDS-PAGE with mass spectrometry to identify the synaptosome protein expression patterns of synaptosome in depression.

Although experimental animal, genomic and proteomic studies are providing some insight into the pathophysiological processes that may be occurring in the depression, at present the molecular correlates underpinning these abnormalities are not fully understood. Proteomics analysis by BN/SDS PAGE combined with tandem mass spectrometry allows simultaneous separation and identification of many hundreds of proteins and protein complexes located in mitochondria and synaptic plasma membrane and is proving to be an effective method to identify the molecular changes associated with depression.

3. Conclusion

This chapter has presented a new proteomic approach to identify the molecular changes associated with depression. BN/SDS PAGE together with other techniques allows the large-scale study and analysis of plasma membrane protein complexes and identifies differentially expressed proteins and protein complexes in large-scale, which serves as a powerful and promising mechanism for extracting useful knowledge and reaching interesting biological conclusions. This technique is enriched with new applications which focus on detecting previously unknown protein functions and relations in depression, which can offer significant experimental knowledge to experts in drug design and clinical applications. BN-PAGE might be the ideal tool to begin study of new protein complexes in brain and search for the differentially expressed proteins and protein complexes in various diseases and gain a deeper understanding of the unique aspects of protein-protein interactions in depression cellular processes.

4. Acknowledgment

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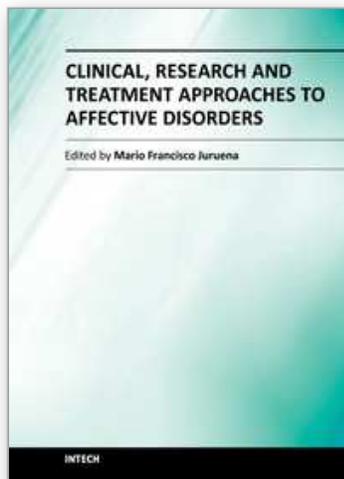
Clinical, Research and Treatment Approaches to Affective Disorders

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The causes, development and outcomes of disorders are determined by the relationship of psychological, social and cultural factors with biochemistry and physiology. Biochemistry and physiology are not disconnected and different from the rest of our experiences and life events. This system is based on current studies that report that the brain and its cognitive processes show a fantastic synchronization. Written by the foremost experts on Affective Disorders worldwide, this book is characterized by its innovative, refreshing, and highly sensitive perspective on current knowledge of diagnostic, neurobiology, early life stress and treatment of Mood Disorders. The authors share a deep understanding of unique challenges and difficulties involved in Affective Disorders, and have achieved a balance among clinical, research and new treatment approaches to Affective Disorders. The chapters are written in a comprehensive, easily readable, and highly accessible style, stimulating readers, clinicians and researchers.

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