Proteomics in neuropsychiatric disorders

Christian Rohlff
Oxford GlycoSciences, Abingdon Science Park, Abingdon, UK

Abstract
Assessing human cerebrospinal fluid (CSF) provides a practical way to conduct longitudinal molecular analyses of changes during the course of neurological disease. Integrated and parallel analyses of neurotransmitters, neuropeptides and proteins in CSF may reveal better insights into complex interaction of numerous cell types in the central nervous system (CNS) at an unprecedented level of complexity and detail. Intricate molecular fingerprints of CSF proteins may pinpoint multiple underlying pathogenic mechanisms as well as an acute and a chronic CNS disease component. Some of these changes may be mapped to altered protein expression patterns in clinically relevant cell populations with a causative or diagnostic disease link. A CNS proteome database of primary human CNS tissues may avoid ambiguities of experimental models and accelerate pre- and clinical development of more specific diagnostic and prognostic disease markers and new selective therapeutics.

Received 23 July 2000; Reviewed 24 September 2000; Revised 20 October 2000; Accepted 31 October 2000

Key words: Brain, cerebrospinal fluid (CSF), proteomics, psychiatric disorders.

Introduction
In the majority of psychiatric disorders, little knowledge exists about cellular and molecular abnormalities and their relationship to nervous system structure and function. More precise molecular characterization of abnormalities is a prerequisite for the improved diagnosis, management and treatment of neuropsychiatric disorders. Proteomics is of increasing relevance to clinical neuroscience for several reasons. First, many of the major psychiatric disorders are quite complex with both oligogenic and environmental factors contributing to disease aetiology. Recent genetic linkage studies have identified multiple susceptibility loci for schizophrenia and bipolar disorder and provided evidence for shared genetic susceptibility (Berrettini, 2000; Gershon, 2000). Proteomics technologies provide a complementary strategy to study the functional output of a disease-associated gene abnormality, irrespective of how strongly genetic the illnesses are. These changes are likely to expand into changes in other proteins resulting in a new protein expression profile. Furthermore, volumetric losses in the cerebral hemisphere and changes in physiological and neuropsychological performance deficits, such as a decreased prefrontal regional cerebral blood flow in twin studies, suggest a significant contribution of cellular and molecular abnormalities to the pathogenesis of schizophrenia (Goldberg, 1994). Proteomics measures all resulting disease-associated changes in proteins as the functional entities of genes simultaneously. Since the vast majority of drug targets are proteins, identification and characterization of a novel molecular target will be greatly facilitated by carrying out the analysis at the protein level. Secondly, no central nervous system (CNS) tissue necessary for any gene expression analysis can be obtained in a living patient under normal circumstances. Proteomic approaches appear more suitable for a molecular dissection of such disease phenotypes in the CNS. The entire CNS is largely inaccessible to meaningful mRNA expression-based analyses of primary human material, since post-mortem delays in primary human brain tissue affects mRNAs more readily than proteins (Edgar et al., 1999). Furthermore, it is becoming apparent that purely gene-based expression analysis is not sufficient for the target discovery and validation process. Gene transcript and actual protein expression are often not unambiguously linked and may be regulated separately (Gygi et al., 1999a). Multiple steps of gene expression can be regulated through stability and turnover of mRNA and protein, post-transcriptional mRNA splicing yielding various protein products as well as further protein diversity through post-translational modifications (PTMs). The physiological and therapeutic importance of distinct splice variant isoforms of a protein has been suggested for 7-transmembrane G protein-coupled receptors (Kilpatrick et
Figure 1. Genome to proteome. Gene expression can be regulated through the rate of transcription, translation, once further post-translational regulation of protein expression and activity are known (Zong et al., 1999). Each protein may undergo various levels of post-translational modifications (PTMs). In some instances, more than 10 serine-, threonine- and tyrosine-phosphorylated and serine- or threonine-O-linked glycosylated amino acids can be detected on a single protein. Additional PTMs include asparagine-linked glycosylation, farnecylation, palmitoylation, all of which can affect the activity, stability and location of a particular protein. There are an increasing number of examples, where enzymes carrying out these PTMs become targets for therapeutic intervention (Parekh and Rohlff, 1997). (Figure 1 from Parekh and Lyall, In Press.)

Proteomics avoids many ambiguities of mRNA gene expression analyses and takes these variables in consideration by focusing on the protein as the ‘biological effector molecule’ of the gene (Gygi et al., 1999b). Additional information becomes available through proteomic analyses of subcellular fractions and protein complexes (see Figure 1). Thirdly, although psychiatric disorders such as schizophrenia and bipolar illness are separate disorders, family studies also suggest some symptom complex overlap (Berrettini, 2000). These genetic changes may reveal aberrant protein mechanisms and protein pathways that may aid subtyping individuals through biological means. A comparative analysis between several neuropsychiatric disorders may lead to the identification of a set of molecular targets and matching biomarkers for more specific diagnostic and prognostic disease markers and new, more selective therapeutic strategies. While proteomics promises an unprecedented and comprehensive means to identify disease-specific proteins, current proteomic technologies (HPLC–mass spectrometry, gel electrophoresis–mass spectrometry) have certain limitations which must be considered before they are applied to provide insight into the protein basis of these diseases.

Neurotransmitters, neuropeptides and proteins in human: an integrated CSF molecular and proteome database for clinical neuroscience

Human body fluids such as cerebrospinal fluid (CSF) and serum can be analysed at the time of presentation and throughout the course of the disease and thus provide clues to molecular pathogenesis. Given that the CSF bathes the brain, changes in its protein composition may reveal alterations in CNS proteins that are causatively or diagnostically linked to the disease. Reasonable amounts of disease-associated proteins (DSPs) are secreted or released into body fluids by diseased tissue in the living patient at the onset of and during the progression of disease. Many of these changes in protein expression will often be independent of the genetic make up of the individual, but also relate to the epigenetic changes in a set of cellular signals, e.g. to changes in gene expression which collectively contribute to the disease phenotype (Carpenter et al., 1998).

Proteomic technologies currently based on 1-D and 2-D gel electrophoresis combined with mass spectrometry have recently progressed dramatically and found increasing applications in molecular medicine (Parekh, 1999;
Proteomics in neuropsychiatric disorders

Disease-specific proteins recently characterized in human CSF from patients with neuropsychiatric disorders

<table>
<thead>
<tr>
<th>CSF analyte</th>
<th>Disorder</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF glutamate</td>
<td>Schizophrenic/schizoaffective patients</td>
<td>Faustman et al. (1999)</td>
</tr>
<tr>
<td>CSF neurotransmitter metabolites: serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) and dopamine metabolite, homovanillnic acid (HVA)</td>
<td>Unipolar depression, schizophrenia and suicide</td>
<td>Asberg (1997)</td>
</tr>
<tr>
<td>Prostaglandins and corticotropin-releasing factor</td>
<td>Schizophrenia</td>
<td>Nishino et al. (1998)</td>
</tr>
<tr>
<td>CSF neuroactive steroids: pregnenolone, progesterone, and neuropeptide diazepam-binding inhibitor (DBI)</td>
<td>Affective disorders</td>
<td>George et al. (1994)</td>
</tr>
<tr>
<td>CSF cannabinoids</td>
<td>Schizophrenia</td>
<td>Leweke et al. (1999)</td>
</tr>
<tr>
<td>Neuropeptides, somatostatin, opioid peptides, vasopressin, neurotensin, neuropeptide Y, substance P and hormones</td>
<td>Depression</td>
<td>Banki et al. (1988); Davis et al. (1988); Sicel et al. (1995)</td>
</tr>
<tr>
<td>Neurotrophin-3</td>
<td>Schizophrenia</td>
<td>Gilmore et al. (1997)</td>
</tr>
<tr>
<td>IL-1, alpha and IL-2</td>
<td>Schizophrenia</td>
<td>Rapaport et al. (1997)</td>
</tr>
<tr>
<td>IL-1β, IL-6 and TNFβ</td>
<td>Schizophrenia</td>
<td>Levine et al. (1997)</td>
</tr>
<tr>
<td>CSF chromogranin A and B, but not C</td>
<td>Schizophrenia</td>
<td>Landen et al. (1999)</td>
</tr>
<tr>
<td>CSF angiotensin-converting enzyme (ACE)</td>
<td>Schizophrenia</td>
<td>Wahlbeek et al. (2000)</td>
</tr>
<tr>
<td>CSF neuronal cell adhesion molecule (N-CAM)</td>
<td>Schizophrenia</td>
<td>van Kammen et al. (1998)</td>
</tr>
<tr>
<td>CSF neuronal cell adhesion molecule (N-CAM)</td>
<td>Mood disorders</td>
<td>Poltorak et al. (1997)</td>
</tr>
<tr>
<td>CSF inositol monophosphatase</td>
<td>Depression and neuroleptic-treated schizophrenia</td>
<td>Atack et al. (1998)</td>
</tr>
<tr>
<td>CSF IL-6 and the soluble IL-6 receptor</td>
<td>Depression</td>
<td>Stubner et al. (1999)</td>
</tr>
<tr>
<td>CSF SNAP-25, GAP-43</td>
<td>Schizophrenia</td>
<td>Thompson et al. (1999)</td>
</tr>
<tr>
<td>α-2 haptoglobin</td>
<td>Schizophrenia</td>
<td>Johnson et al. (1992)</td>
</tr>
<tr>
<td>DBI</td>
<td>Alzheimer’s disease</td>
<td>Ferrarese et al. (1990)</td>
</tr>
<tr>
<td>Aβ42/amyloid precursor proteins</td>
<td>Parkinson’s, Alzheimer’s, and Huntington’s disease</td>
<td>Hock et al. (1998)</td>
</tr>
<tr>
<td>Amyloid precursor protein</td>
<td>Depression/therapy lithium therapy</td>
<td>Clarke at al. (1993a,b)</td>
</tr>
<tr>
<td>Tau/IL-6 receptor</td>
<td>Alzheimer’s disease</td>
<td>Hampel et al. (1999)</td>
</tr>
</tbody>
</table>

Parekh and Lyall, 2000; Rohlf, 2000). Tandem mass spectrometry can be applied simultaneously for the analysis of endogenous neurotransmitters and neuropeptides (Jaeverfalk-Hoyes et al., 1999) as well as for the rapid identification of DSPs (Parekh, 1999; Parekh and Lyall, 2000; Rohlf, 2000). Numerous alterations in neurotransmitters, neuropeptides and proteins documented in psychiatric disorders in many independent studies, some of which are listed in Table 1, signify the relevance of such approaches. Integrated proteomic tools and databases may yield a more comprehensive understanding of neuropsychiatric disorders through a simultaneous measurement of neurotransmitter, neuropeptide and protein expression changes in a diseased brain. Human CSF becomes a rich source of important mechanistic information on a disease phenotype and a unique opportunity to obtain important descriptive information in the patient at the time of disease manifestation.

A routine proteomic application for a rapid and reliable identification of disease-specific protein markers in body fluids has previously been severely limited by the interference by high-abundance proteins such as ‘albumin’, ‘haptoglobin’, ‘IgG’ and ‘transferrin’. For example, without depletion of the high-abundance proteins, less than 1000 individual features could be separated in CSF (Yun et al., 1992) (see Figure 2a,b). The preparation of protein in CSF for proteomic analyses has been significantly improved through immunoaffinity-based depletion methods (Rohlf, unpublished results). After such an enrichment, many additional features previously masked by the high-abundance proteins become visible (Figure 2c,d) and up to approx. 2500 features of a CSF...
Protein expression mapping aims to define global protein expression profiles in tissues, cells or body fluids. The most common implementation of proteomics is based on 2-D gel electrophoresis of proteins in a complex mixture and their subsequent individual isolation, identification and analysis from within the gel by mass spectrometry. The CSF proteins are solubilized in 2-D gel electrophoresis sample buffers and run on 2-D polyacrylamide gels by separating them according to their charge (pI) in the first dimension using isoelectric focusing. They are then separated by size, using SDS–PAGE, in the second dimension. Fluorescent dyes, that bind non-covalently to SDS-coated proteins, enable the detection of protein spots in the gel over a greater linear range than densitometric methods, while not interfering during the mass spectrometry analysis (Page et al., 1999). A routine application of this approach for a rapid and reliable identification of disease-specific protein markers had been severely limited by the interference of high-abundance proteins such as albumin, haptoglobin, IgG and transferrin. For example, without removal of these high-abundance proteins, less than 1000 individual features can be separated in CSF. A refined use of proteomics for these types of analyses through immunoaffinity-based depletion methods in which four highly abundant proteins are removed prior to analysis. After such enrichment, many additional features previously masked by the high-abundance proteins become visible.

For example, changes in DSP expression which occur during neuronal loss and impaired synaptogenesis, a hallmark of many neuropsychiatric disorders, subsequently result in disease-specific alterations of neuronal and CSF proteins. Synaptic pathologies have been implicated in several neuropsychiatric disorders including schizophrenia (Heinonen et al., 1995). Consequently, it is not surprising that changes in synaptic proteins such as SNAP-25 (Thompson et al., 1999), neurotensin (Sharma et al., 1997) and N-CAM (van Kammen et al., 1998; Vawter et al., 1998) have been detected in the CSF of schizophrenia patients. Significance can be attributed to these results, since N-CAM levels are changed in affected twins but not in healthy siblings (Poltorak et al., 1997) (see Table 1), supporting a link of this molecular event to the sample can be separated through 2-D gel electrophoresis on a single pH 3–10 gradient and analysed for disease-specific changes in protein expression.
pathogenesis of schizophrenia. Such DSPs may provide important insights into disease pathology and opportunities for better diagnosis and treatment strategies. However, in some cases these changes are specific to a single disorder, such as the elevation of α-2 haptoglobin in schizophrenia and Alzheimer’s disease (Johnson et al., 1992), and elevated SNAP-25 levels in schizophrenia and bipolar patients (Thompson et al., 1999). Recent advances in proteomic bioinformatics can test a repertoire of DSPs for their specificity and sensitivity over several neurological disorders as well as acute and chronic CNS disease. Consequently, comprehensive proteomic CSF databases may grant us this opportunity and map out complex interactions of many cell types relevant to the disorder at an unprecedented level of detail through a simultaneous description of changes at the protein, neuropeptide and neurotransmitter level both in CSF and CNS tissue. A number of DSPs such as amyloid precursor protein, β-amylloid, tau, presenilin-2 and apolipoprotein-E have also been characterized in plasma of Alzheimer’s patients (Ueno et al., 2000) or serum creatine kinase and aldolase abnormalities in patients with psychoses (Meltzer, 1998). Changes in serum concentrations of a N-CAM fragment (Lyons et al., 1988) and apolipoprotein-E allele 2 in untreated schizophrenia patients (Kimura et al., 1997) and soluble IL-2 and IL-6 receptors in serum of neuroleptic-treated schizophrenia patients (Müller et al., 1997) have also been documented. Therefore, the expression of DSPs, identified in CSF in readily accessible peripheral body fluids such as plasma and serum, may yield useful diagnostic and prognostic biomarkers. CSF could be considered a complementary ‘compartment’ to CNS cells and tissue and as such provides a means to monitor in a longitudinal manner underlying changes in neuronal cells.

CNS tissue proteomics – a link between cellular and molecular abnormalities and disease phenotype

Insights gained from proteomic CSF studies should ideally be extended to the cellular level. A proteomic identification of disease-specific molecular changes in affected brain areas implicated in the pathology will be necessary to find novel therapeutic modalities that correct dysfunctional cellular processes. The feasibility of such an approach has also been exemplified in studies on synaptosomal proteins in CSF and CNS tissues by Davidsson et al. (1999), Greber et al. (1999) and glial fibrillary acidic protein (GFAP) isoforms in the frontal cortex of schizophrenia, bipolar disorder, and major depression patients (Johnston-Wilson et al., 2000) and others (Table 2). Emerging new technologies such as laser capture microdissection (LCM) (for review see Sirivatanauksorn et al., 1999) are able to provide cellular definition within complex human tissues for proteomic analyses (Banks et al., 1999), PCR analysis (Emmert-Buck et al., 1996) and cDNA microarray gene expression profiles (Luo et al., 1999).

In addition, subcellular fractionation and enrichment strategies such as a membrane protein purification increase the likelihood of detecting low-abundance proteins and simultaneously study their subcellular distribution. Such approaches to improve the proteomic detection of membrane proteins (Rabilloud et al., 1997; Vuillard et al., 1995) have been further optimized for human cerebellar cell membranes through additional salt and urea wash enrichment steps (Frizzo and Wikström, 1999). Alternatively, functional groups in a given family of proteins may provide the right molecular hook to allow for the analysis of this particular protein class using affinity columns such as heparin chromatography, used for the enhanced detection of low-abundance protein in human brain extracts (Karlson et al., 1999).

While the tissue analysis will provide a more detailed characterization of the molecular changes occurring in affected brain regions at the time of the patient’s death, they will not yield any information about changes in protein expression during disease onset and progression. This re-emphasizes the value of CSF protein expression profiles obtained from a living patient during disease presentation and/or progression.

Identities of disease-specific protein isoforms resulting from post-translational modifications (PTMs) provide opportunities for new therapeutic approaches

Cellular processes and inter- and intra-cellular signalling cascades are altered in the majority of CNS disorders. The proteins and protein complexes involved in these processes are regulated in many cases through PTMs and many enzymes carrying out these PTMs are being evaluated as targets for novel modalities of therapeutic intervention in many areas, including neuronal abnormalities (Manji et al., 1999a; McShea et al., 1999; Parekh and Rohlf, 1997). Rational design of modulators of these new molecular targets has yielded multiple drug candidates, promising more selective therapies. Some receptor tyrosine kinases (RTK) mediate cellular stress by activation of G protein-coupled receptors (Hackel et al., 1999). Downstream signalling molecules such as protein kinase C (PKC) and MAP kinase have been implicated in CNS disorders such as depression (Ikonomov and Manji, 1999) and Alzheimer’s disease, where a recent study by Hensley et al. (1999) suggests that p38 MAP kinase is activated in brain extracts from patients with Alzheimer’s disease. The latter may be due in part to increased
Disease-specific proteins recently characterized in human CSF and CNS tissues from patients with neuropsychiatric disorders

<table>
<thead>
<tr>
<th>Tissue protein</th>
<th>Indication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAP-25/GAP-43*</td>
<td>Schizophrenia</td>
<td>Blennow et al. (1999), Greber et al. (1999)</td>
</tr>
<tr>
<td>SNAP-25/GFAP</td>
<td>Alzheimer’s disease, Down Syndrome</td>
<td>Edgar et al. (1999)</td>
</tr>
<tr>
<td>DBI*</td>
<td>Alzheimer’s disease, schizophrenia</td>
<td>Edgar et al. (2000)</td>
</tr>
<tr>
<td>Tau*</td>
<td>Alzheimer’s disease</td>
<td>Hensley et al. (1999)</td>
</tr>
<tr>
<td>Hippocampal DBI*, manganese superoxide dismutase, T-complex protein 1, collapsin response mediator protein 2</td>
<td>Schizophrenia</td>
<td>Blennow et al. (1999)</td>
</tr>
<tr>
<td>cAMP regulatory element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF)</td>
<td>Schizophrenia and bipolar disorder</td>
<td>Jonhston-Wilson et al. (2000)</td>
</tr>
<tr>
<td>Frontal cortex GFAP</td>
<td>Schizophrenia, bipolar disorder, and major depression</td>
<td>Jonhston-Wilson et al. (2000)</td>
</tr>
<tr>
<td>Frontal cortex nicotinic receptor alpha7 subunit</td>
<td>Schizophrenia</td>
<td>Guan et al. (1999)</td>
</tr>
<tr>
<td>Cerebellar vermis mitogen-activated protein kinases</td>
<td>Schizophrenia</td>
<td>Kyosseva et al. (1999)</td>
</tr>
<tr>
<td>Hippocampal (CA3) NMDA receptors</td>
<td>Schizophrenia</td>
<td>Dean et al. (1999)</td>
</tr>
<tr>
<td>Serotonin_{3a} receptors in Brodmann’s area 9</td>
<td>Schizophrenia</td>
<td>Dean et al. (1998)</td>
</tr>
</tbody>
</table>

*Proteins have also been measured in CSF.

oxidative stress (Perry et al., 1999), and a differential activation of small G proteins (Shimohama et al., 1999).

Thus, the evidence-driven rationale of small molecule inhibitors in complex CNS diseases may now be going beyond the classical approaches of neuropsychopharmacology and hold new promise in the search for more selective and efficacious treatments (Liebermann, 2000). Recent examples of these efforts include PKC inhibitor strategies for the treatment of major depression and preclinical evaluation of MAP kinase inhibitors for post-ischaemic brain injury (Alessandrini et al., 1999; Manji et al., 1999a). In addition to providing a rationale for novel therapeutic approaches, proteomic-based studies may expand the knowledge mechanism of action for established therapies, guiding the scientist to better second-generation compounds. For example, the anti-

![Figure 3. Proteomic applications in CNS research for accelerated drug discovery (Figure 3 from Rohlff, 2000).](Image)
manic efficacy of valproic acid and lithium in manic-depressive illness has now been linked to down-regulation of PKC in vitro (Chen et al., 1994), and increase of the neuroprotective protein bcl-2 in the CNS occurs in recognized animal models in the case of lithium (Chen et al., 1999). Considerable literature supports the relevance of these complex molecular and cellular mechanisms for lithium’s neuroprotective effects in mood disorders. These neuroprotective activities appear important for the therapeutic benefits of these drugs, since significant reductions in regional CNS volume and cell numbers (both neurons and glia) are associated with many CNS diseases including mood disorders (Manji et al., 1999b). Finally, preclinical evaluation of candidate targets and compounds requires complex animal models that will represent as many of the aspects of the human disease as possible. Evaluating such models is often not simple. The recapitulation of the changes in protein expression found in clinical samples compared to animal models of disease provide a firm molecular assessment of these models (Figure 3).

Conclusion – CNS proteomics in accelerated drug development

Proteomic analysis of human CSF provides important mechanistic information on a disease phenotype at the time of disease manifestation. The value of the data may be improved through a comparative analysis between several CNS diseases for more specific diagnostic and prognostic disease markers and new, more selective therapeutic strategies.

Enhanced integrated proteomic databases can assign up to several hundred DSPs in complex body fluids and purified authentic human tissues with statistical confidence combined with parallel datasets on neurotransmitters, neuropeptides and DSPs. An increasing number of disease-specific candidate target proteins relevant at the presentation of the disease can be matched to diagnostic and prognostic proteomic markers, to accelerate preclinical and early stage of clinical drug development (Figure 3). Together with other emerging technologies such as protein chip arrays (Senior, 1999) the utility of body-fluid-derived proteomic markers may further expand in later stages of drug development. A rapid increase in proteomic technology sensitivity and throughput combined with a new generation of bioinformatics tools (Parekh and Lyall, In Press) facilitates the evaluation of complex biological systems at great speed. Such comprehensive analysis may increase our confidence to pursue novel, more selective, and efficacious therapeutic strategies for neuropsychiatric disorders.

Acknowledgement

Thanks are due to thank Jim Bruce and Robin Philp for the images of human CSF and to Dr Raj Parekh for his helpful comments on the manuscript.

References


Chen G, Zeng WZ, Yuan PX, Huang LD, Jiang YM, Zhao ZH, Manj HK (1999). The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *Journal of Neurochemistry* 72, 879–882.


bipolar disorder, and major depressive disorder. Molecular Psychiatry 5, 142–149.


Manji HK, Moore GJ, Chen G (1999b). Lithium at 50: have the neuroprotective effects of this unique cation been overlooked? Biological Psychiatry 46, 929–940.


Srivatanauksorn Y, Drury R, Crnogorac-Jurcevic T,


Further studies of elevated cerebrospinal fluid neuronal cell adhesion molecule in schizophrenia. *Biological Psychiatry* 43, 680–686.


