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The Role of Energy Metabolism Dysfunction and Oxidative Stress in Schizophrenia Revealed by Proteomics

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Abstract

Schizophrenia is a psychiatric illness that affects approximately 30 million people worldwide. Converging lines of evidence suggest that mitochondrial function may be compromised in this disorder, and this can lead to perturbations in calcium buffering, oxidative phosphorylation, increased production of reactive oxygen species, and apoptotic factors, which can, in turn, affect neuronal processes such as neurotransmitter synthesis and synaptic plasticity. Proteomics studies in brain and peripheral tissues of schizophrenia patients have provided considerable evidence and identified biomarker fingerprints corresponding to such pathways. Here we review the results of these studies with a focus on the biomarker pattern depicting alterations in energy metabolism and oxidative stress in this debilitating illness. *Antioxid. Redox Signal.* 15, 2067–2079.

Introduction

Proteomics

Definition. The term "proteome" was defined originally as "the study of the total set of expressed proteins by a cell, tissue, or organism at a given time under a determined condition" (86). The term now has a wider meaning, as it also includes the posttranslational modifications made to a particular set of proteins produced by an organism or cellular system, which may vary dynamically with time or because of distinct changes in environmental factors. Proteomic technologies allow the identification of small to large subsets of proteins that are associated with these differences.

Methods

The most traditional proteomic method is a combination of two-dimensional gel electrophoresis (2DE) for protein separation and mass spectrometry (MS) for identification. This combination of technologies allows the simultaneous separation of many hundreds of proteins in a single experiment. Proteins are first separated by gel electrophoresis in two dimensions according to physical properties such as isoelectric point and apparent molecular weight. Gels are stained by using, for instance, coomassie blue or silver nitrate; gel images are matched, and differentially expressed proteins are identified by MS fingerprinting or sequencing. Pre-electrophoretic fluorimetric methods such as fluorescent 2-DE difference gel electrophoresis (2D-DIGE) have also been used for proteome analyses; these require only a single gel to detect reproducible proteome differences and low amounts of sample (detection limit, between 150 and 500 pg). In 2D-DIGE experiments, the samples to be compared are first labeled by using distinct cyanine-derived fluorophores, which are mass and charge matched (termed Cy2, Cy3, and Cy5), and then these labeled samples are run in the same gel, thereby minimizing technical variation. The fluorophores interact with the free amines on proteins, allowing the comparison of two samples plus an internal standard for normalization purposes and multigel comparisons. Gels are digitalized in a fluorescence scanner, revealing the spot volumes of the individual samples. The spot volumes of each sample are correlated to the internal standard, providing a volume ratio between sample and internal standard spots. The relative abundances of the spots in distinct samples are then compared, revealing the differentially expressed proteins (50).

Despite the separation power of this approach, 2DE- or 2D-DIGE-MS–based proteomics has some limitations, such as an inherent difficulty in detecting integral membrane proteins, as well as proteins with extremes of isoelectric point or molecular weight (32). Such drawbacks have led researchers to explore additional methods of identifying these troublesome classes of proteins.

Direct MS-based approaches have also been used, known as shotgun proteomics or liquid chromatography–tandem mass spectrometry (LC-MS/MS), which use distinct chromatographic separation before the MS stage (58). Basically in LC-MS/MS methods, the proteins under comparison are first

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digested enzymatically to produce peptides, which are normally labeled with the different forms of stable isotopes such as ICAT (isotope-coded affinity tags), iTRAQ (isobaric tag for relative and absolute quantitation), or ICPL (isotope-coded protein labeling) for posterior quantification. The labeled peptides are then separated with chromatography before identification and quantification with MS. Label-free LC-MS/MS approaches can also be optimized, which would be more suitable for biomarker discovery in larger sample sets, because proteome analyses would not be limited to the number of isotopes for quantification (56). However, because these analyses are carried out at the peptide level, a potential drawback is that they do not provide direct information on intact proteins, as is the case for 2DE methods.

Whereas 2DE-MS and LC-MS/MS approaches have been used to reveal the global protein expression of a given tissue, other methods such as Western blot (WB), immunoadsorbent assay, multiple reaction monitoring MS, and multiplex immunoassay are used commonly for the validation of differentially expressed proteins. This is essential because it must be demonstrated that the differences found in a limited small set of samples also can be identified in a broader range of samples. Moreover, implementation and improvement of high-throughput methods for validation studies and for the translation of the measurements to a clinically useful format are still required. A convenient, rapid, sensitive, and specific solution might be the fluorescent bead–based technologies, which allow multiplex analysis in individual small-volume samples. Such approaches have already been used successfully in clinical studies of epithelial ovarian cancer (8), scleroderma (19), coronary artery disease (31), myocardial infarction (20), autoimmune disorders (17), and sickle cell anemia (55). However, this method has been implemented only recently in studies of brain disorders (12). In brief, the technology uses multiplexed dye-coded microspheres, coated with specific capture reagents for assaying targeted analytes within clinical samples. Identification and quantitation of the analytes is achieved with laser excitement within an analyzer. The format allows multiplexing of up to 100 distinct assays for a single sample. The several advantages to this approach include the minimization of sampling errors, lower amounts of sample required per analysis, and the reduced costs of assay reagents.

Because all of the methods described here have their merits and weaknesses, the combined use of two or more of these would be best to maximize coverage of the relevant molecular pathways (Fig. 1).

Schizophrenia

Schizophrenia (SZ) is a debilitating mental disorder that affects about 30 million people worldwide, with men and women being equally affected. It is characterized by positive symptoms, such as hallucinations and delusions, negative symptoms, such as anhedonia, and by cognitive deficits. The current methods of diagnosis are subjective, as these are based on psychiatric interviews. At the neuropathologic level, SZ has no single diagnostic feature. In general, it appears to be characterized by synaptic deficits, particularly in the dorsolateral prefrontal cortex (DLPFC) and hippocampus (35), alterations in glutamate and dopamine neurotransmission (15, 43), and hypofrontality (87). The onset and development of SZ is believed widely to be the result of interactions between multiple genes and environmental factors, which lead to impaired neurodevelopment and manifestation of the disease, normally in late adolescence or early adulthood.

Perturbations in glucose handling: the evidence

An early report on the incidence of impaired energy metabolism in SZ was published approximately 90 years ago (51). In an extensive analysis of different psychiatric disorders, Kooy (38, 83) observed hyperglycemia in SZ patients, similar to the effects seen in *dementia praecox* subjects. Kooy hypothesized that depression-like behavior influences the levels of blood sugar. However, it is still not known whether high blood sugar in psychiatric illnesses is a cause or a consequence of the disease.

Alterations in glucose handling in the SZ brain have been demonstrated by several studies. A lower rate of glucose metabolism was detected in the hippocampus and the anterior cingulate cortex (ACC) of SZ patients compared with controls (82). Conversely, administration of glucose to SZ patients resulted in increased verbal declarative memory and attention (25).

Over the last 10-year period, numerous studies demonstrated the occurrence of hyperglycemia, impaired glucose tolerance, and/or insulin resistance in first-onset, antipsychotic, naïve SZ patients (23, 24, 29, 75, 79, 84, 85).

Imaging studies have also revealed an impairment of energy metabolism in SZ brains. By using a rheoencephalography study of 30 SZ patients and 20 controls, Jacquy and colleagues in 1976 (45) observed lowered cerebral blood flow in the frontal cortex. This marked the first occasion in which the term "hypofrontality" was used in SZ research. The general findings were supported in 1985, when positron emission tomography (PET) studies using deoxyglucose revealed that SZ patients had lower metabolic rates in their frontal brain region before and even after the treatment with antipsychotics (88). Andreasen and co-workers (2) gave support to the observations of Kooy, by using magnetic resonance imaging (MRI) to confirm that cerebral energy metabolism was impaired in SZ. Shortly afterward, these findings were linked to a differential dopaminergic state (28) and to perturbed phospholipid breakdown (26). Both of these effects are thought to be important features in the etiology of SZ. In addition, the observation of impaired energy metabolism reinforced the concept that mitochondrial metabolism is perturbed in SZ (reviewed in 7).

Mitochondrial dysfunction

Mitochondria produce about 95% of the energy requirements in eukaryotic cells. These organelles are highly concentrated in the brain because of the high energy demands of this tissue. However, abnormally high mitochondrial concentrations can lead to mitochondrial disorder (9).

Mitochondria were first described by the Swiss scientist Albert von Kölliker as muscular "granules" in 1857. In 1886, Richard Altman described the organelles as "bioblasts," but it was not until 1898 that the term "mitochondrion" was introduced by the German scientist Karl Benda. Since then, a number of studies regarding mitochondria and energy metabolism included the findings of Warburg in 1912, who studied the cellular effects of cyanide and cellular oxygen

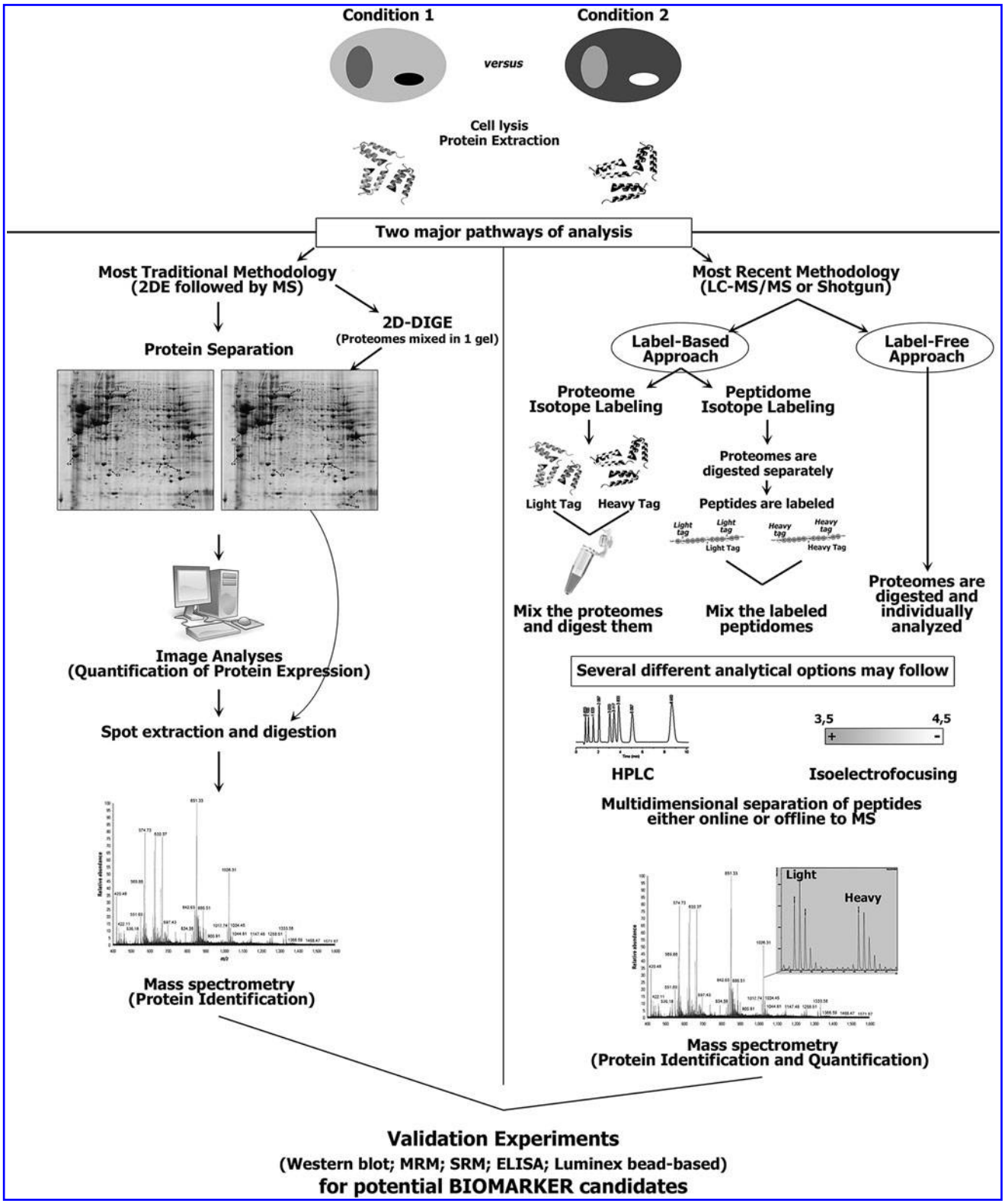

FIG. 1. Basic schema of proteomics method. 2DE, two-dimensional gel electrophoresis; 2DE-DIGE, fluorescent two-dimensional differential gel electrophoresis; MS, mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry (shotgun proteomics); HPLC, high-performance liquid chromatography; MRM, multiple reaction monitoring; SRM, single reaction monitoring; ELISA, enzyme-linked immunosorbent assay.

processing, Keilin in 1923, who studied the function of cytochromes, Fiske and Subbarow in 1929, who isolated ATP molecules, Kennedy and Lehninger in 1950, who demonstrated the process of mitochondrial oxidation, Mitchell in 1978, who described the link between transport of hydrogen ions across mitochondrial membranes in ATP generation, and Boyer in 1979, who described ATP synthase roles in mitochondria.

Glucose is the key molecule for generation of cellular energy, and the process occurs in mitochondria. Observations of impairments in glucose metabolism in SZ, as described earlier, and the role of glucose in generation of redox potential within cells, have led researchers to study mitochondrial dysfunctions in SZ. It is still not clear whether mitochondrial dysfunction or impaired glucose metabolism is affected first in the cascade of events leading to SZ.

Researchers have investigated potential mitochondrial dysfunction as a causative factor in SZ since the 1960s. A number of studies conducted by Russian scientists led to consistent findings (30, 53). Most recently, it has been hypothesized that the broad mitochondrial processes are affected in SZ, such as calcium homeostasis, cell death, neurotransmitter transport, synaptic plasticity, and exacerbated production of reactive oxygen species (ROS) (reviewed in 7). Most probably, higher ROS concentrations are not properly processed in early SZ patients because key processes of stress response, such as the purine catabolism, are disturbed (92).

Mitochondria contain an intricate network of enzyme activities, which are tightly regulated to optimize anabolic and catabolic processes. Any perturbation in activity of one or more of these components could result in altered energy production, which could lead to overproduction of damaging ROS and, in turn, compromise brain function.

SZ is characterized predominantly as a disorder of synaptic connectivity. Mitochondrial oxidative stress leads to DNA damage, malfunction of energy production through protein inactivation, altered gene and protein expression, and finally, apoptosis and cell death. All of these processes can lead to impaired neuronal plasticity and perturbed neurotransmission, along with an increased vulnerability to biologic insults or stress or both. Such processes may start during neurodevelopment in SZ patients and coincide with manifestation of symptoms during early adulthood (6, 90).

Proteomic Evidence of Energy Metabolism Dysfunction and Oxidative Stress

The results of proteomic-profiling studies have led researchers to conclude that a dysfunction of energy metabolism, involving mitochondrial and nonmitochondrial processes, as well as an exacerbating production of ROS, is found in brain tissue of SZ patients. These effects also appear to be reflected in peripheral tissues such as cerebrospinal fluid, red and white blood cells, and tissues such as liver (73, 77).

Glycolysis

Generation of cellular energy starts with the process of glycolysis. Once transported to the cellular cytosol by the facilitated glucose transporters (GLUT receptors), glucose is phosphorylated and processed through the glycolytic enzymatic reactions. The final products of glycolysis are two molecules of pyruvate, which can be converted in mitochondria to acetyl-CoA and oxaloacetate to initiate the Krebs tricarboxylic acid cycle. Proteome analyses of different brain regions from SZ patients have consistently revealed a number of differentially expressed glycolytic enzymes, as shown in Table 1 and in reviewed in (66). The most consistent differentially expressed enzyme is aldolase C (ALDOC), which has been found in eight of 14 proteome analyses. ALDOC reversibly catalyzes the aldol cleavage of fructose-1,6-biphosphate and fructose 1-phosphate to dihydroxyacetone phosphate or glyceraldehyde-3-phosphate or both. The final product of glycolysis, pyruvate, was quantified in the thalamus of SZ patients and found to be present in lower amounts when compared with healthy controls (65). The lower levels of pyruvate in SZ brains suggest potentially compromised function of the Krebs cycle. However, this possibility still requires functional validation.

Although the involvement of glycolysis in SZ appears to be consistent across different proteome reports, some confounding factors must be considered. The analyzed SZ brain tissues were derived from patients who were given long-term treatment with antipsychotics, which may affect protein-expression data. Some glycolytic enzymes, such as aldolase A (ALDOA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and phosphoglycerate mutase 1 (PGAM1) were also found to be differentially expressed in the frontal cortex of olanzapine-treated rats (59). Such findings do not disaffirm what has been found in human brain tissue, but add further information that must be considered. It is not surprising that these enzymes are differentially expressed in both SZ-treated patients and in antipsychotic-treated rat models, as the regulatory systems of these enzymes are sensitive to fluctuation by multiple factors.

Despite the cited confounding factors, the systemic differential expression of glycolytic enzymes led us to consider the likelihood that glucose metabolism in SZ is impaired, as are pathways such as the Krebs cycle and oxidative phosphorylation (OXPHOS), which are closely related. The effects on expression of these metabolic enzymes can result in differential ROS production, considering the transport of vulnerable electrons through these pathways via oxidation and reduction of NAD^+, $NADP^+$ and ADP→ATP production, changing the redox state of SZ tissues and leading to oxidative stress.

Effects on insulin signaling

As cited earlier, proteome analyses of SZ tissues revealed that glucose metabolism is impaired in SZ. This has been supported by previous findings such as an occurrence of impaired glucose tolerance and metabolic syndrome in first-episode drug-naïve SZ patients (68, 79). Impaired glucose metabolism invariably leads to insulin resistance in cells, which can, in turn, lead to increased insulin levels (57). Because many studies using SZ patient samples came from treated subjects, it was hypothesized first that antipsychotic drugs exert an effect on glucose metabolism and the insulin response (40, 57). Indeed, antipsychotic drugs such as clozapine and olanzapine can lead to body-weight gain, type 2 diabetes mellitus, and hyperlipidemia (81). However, it was shown recently that SZ patients can have insulin resistance,

independent of antipsychotic treatment (80). This finding is supported by a parallel study of serum and plasma from four groups of first-onset SZ patients, which found no significant alterations in the glucose levels in three of the groups, although all four groups had elevated rates of insulin-related peptides (29). It is interesting to note that nondiabetic SZ patients who have higher levels of insulin have a better psychopathology profile in the acute disease (21), which may be a clue for more-effective treatments, as is further discussed.

Krebs cycle

The Krebs cycle generates energy, as well as secondary metabolites for other biochemical pathways, such as amino acid production and redox potential for OXPHOS.

The conversion of pyruvate to acetyl-CoA appears to be dysregulated in the prefrontal cortex (PFC) of SZ patients, because proteomic studies have found that expression of pyruvate dehydrogenase E1 component alpha 1 (PDHA1) is decreased in this tissue (72). Also, aconitase 2 (ACO2), which catalyzes the second step of Krebs cycle, showed increased expression in the ACC, DLPFC, and Wernicke area (WA) in SZ patients (4, 61, 64), and expression of this protein, and that of malate dehydrogenase 1 (MDH1), was decreased in the PFC-BA9 (72). One potentially confounding factor that should be considered is that the brain tissue under analysis could have been influenced by the antipsychotic treatments that these patients had undergone. This is especially true, as Krebs cycle enzymes, such as citrate synthase (CS) and malate dehydrogenase 2 (MDH2), have been found to be decreased in the frontal cortex of haloperidol- and olanzapine-treated rats (59).

The role of the Krebs cycle as a producer of redox potential has been tested through quantitation of NADH and NADPH levels. Higher levels of NADH have been found in the thalamus of SZ patients (65), indicating a disturbed production of ion donators, which might compromise OXPHOS.

OXPHOS

Alterations in mitochondrial OXPHOS have been observed consistently in brain tissue and platelets from SZ patients (5, 37). Morphological, volume, and density alterations of mitochondria in SZ have also been demonstrated (reviewed in 7). Proteomic studies have found that several components of the mitochondrial complex I and V are altered in SZ. Complex I is composed of 45 subunits localized in the mitochondrial inner membrane. This complex has NADH dehydrogenase and oxidoreductase activities, which are involved in transfer of electrons from NADH to the respiratory chain. A number of complex I subunits, such as NDUFB5, NDUFB9, NDUFS3, NDUFS6, and NDUFV2, have been found to be downregulated in SZ brain tissue, mainly in the anterior temporal lobe (ATL) (62). NDUFV2 mRNA expression (but not protein expression) has been found to be decreased in rats treated with clozapine and quetiapine (47).

Mitochondrial complex V is a general term for the enzymatic complex that synthesizes ATP, the cellular energy derived from ADP and inorganic phosphate. This complex is formed by 16 subunits divided into two domains termed F0 (membrane domain) and F1 (site of ATP synthesis). In SZ, different ATP synthase subunits, such as ATP5A1 and ATP5H, have been found to be altered in different SZ brain regions (reviewed in 66).

Oxidative stress

As described earlier, oxidative-stress events are present in SZ and have been identified by proteomic technologies. The peroxiredoxins (PRDXs) 1, 2, and 6 have been identified in the PFC and WA from SZ patients. PRDXs reduce H_2O_2 to $H_2O + O_2$ and are regulated by phosphorylation and redox states that protect cells against oxidative injury. Interestingly, PRDXs may also act in phospholipid turnover, which is known to be altered in the pathogenesis of SZ (26, 27). The resulting impaired cellular membrane structure may lead to oxidative-stress conditions.

Three members of glutathione transferases (GST) family (GSTM3, GSTTLp28, and GSTP1) were found to be differentially expressed in SZ thalamus and PFC (65, 72). GSTs are multifunctional enzymes involved in cellular detoxification, glutathione reduction, and neutralization of ROS (36). The main roles of GSTs and their differential expression in SZ brain tissue led to hypotheses that oxidative stress may be involved in the pathogenesis of SZ.

Carbonyl reductase 1 (CBR1), carbonyl reductase 3 (CBR3) and quinoid dihydropteridine reductase (QDPR), which have been found differentially expressed in the PFC and thalamus of SZ patients, are NADPH-dependent oxidoreductases and may be influenced by the oxidative-stress state in SZ tissue. The imbalance of the NADP/NADPH ratio observed in SZ thalamus may have an influence over the expression.

Expression of the antioxidant enzyme superoxide dismutase [Cu-Zn] (SOD1), which dismutates the superoxide anions into oxygen and hydrogen peroxide, was found to be decreased in the liver of SZ patients, probably as a response to dysregulation of free radical metabolism. This protein was also shown to be altered in red blood cells (RBCs) of SZ patients (73).

Oxidative stress as a central feature of SZ

Results from animal-model studies suggest that oxidative stress may be a central feature of SZ. The psychotomimetic properties of phencyclidine (PCP) may induce cognitive dysfunction in healthy subjects (14), exacerbate psychoses in SZ patients, and induce positive and negative SZ symptoms in rodents (46). One of the biochemical mechanisms involved in the PCP action seems to be generation of oxidative stress, because rats treated over the long term with PCP had changes in the antioxidant mechanisms, as seen by alteration of glutathione peroxidase (GPx), glutathione reductase (GR), and SOD activities in the DLPFC. Moreover, decreased activities of GPx and GR were found in the hippocampus, along with diminished concentration of reduced GSH in both brain regions (74).

Lipid metabolism

More than half of the brain's dry weight is composed of lipids, which play a structural role in cellular membranes and are also integral to the process of neuronal transmission. Effects on lipids in SZ have been described previously (42), including an increased phospholipid turnover in the frontal lobe and thalamus (27, 76) and decreased levels of phosphatidylcholine and docosapentaenoic acid in the

Table 1. Differentially Expressed Proteins Related to Energy Metabolism in Schizophrenia Brain Tissue: The Most Consistent Findings

Energy metabolism process	*Gene name*	*Protein name*	*MW (th)*	*pI (th)*	*BA10 2DE*	*BA9 2DE-DIGE*	*BA24 2DE*	*BA24 2DE**	*CC 2DE*	*BA9 2DE*	*IC 2 DE-DIGE*	*BA46 2DE*	*BA38 Shotgun*	*BA46 Shotgun*	*BA22 2DE*	*Thalamus shotgun 2DE*
Glycolysis	ALDOC	Fructose bisphosphate; aldolase C	39455.9	6.41	↑	↓	↓				↑	↑	↓		↓	
Glycolysis	ENO2	Gamma enolase (2-phospho-D-glycerate hydro-lyase)	47137.8	4.91		↓			↓	↓					↑	
Glycolysis	HK1	Hexokinase brain form	102484.9	6.44		↑							↑	↑		
Glycolysis	PGAM1	Phosphoglycerate mutase 1	28673.0	6.75		↓					↓				↑	↑
Glycolysis	TPI1	Triosephosphate isomerase	26538.5	6.51		↓					↓				↑	↑
Glycolysis	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	35922.3	8.58		↓									↓	↑
Glycolysis	PGK1	Phosphoglycerate kinase 1	44615.1	8.30								↑				
Krebs cycle	ACO2	Aconitate hydratase, mitochondrial precursor (aconitase)	85426.1	7.36		↓		↑				↑			↑	
Krebs cycle	MDH1	Malate dehydrogenase 1, NAD (soluble)	36426.2	6.91		↓						↓				
(Pre) Krebs cycle	PDHA1	Pyruvate dehydrogenase E1 component, alpha 1	43295.7	8.35		↓										
(Pre) Krebs cycle	DLD	Dihydrolipoyl dehydrogenase, mitochondrial precursor	54150.7	7.59											↑	
(Pre) Krebs cycle	ME3	Malic enzyme 3	67068.5	8.16												↑
OXPHOS	ATP5A1	ATP synthase alpha chain, mitochondrial precursor	59751.1	9.16								↑			↓	
OXPHOS	ATP5H	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform a	18491	5.21								↓				
OXPHOS	COII	Cytochrome *c* oxidase subunit 2	25565.0	4.67												↓
OXPHOS	NDUFB9	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9	21830.9	8.57												↑
OXPHOS	NDUFS6	NADH-ubiquinone oxidoreductase 13-kDa-A subunit	13711.6	8.58									↓			
OXPHOS	NDUFB5	NADH-ubiquinone oxidoreductase SGDH subunit	21750.3	9.62									↓			
OXPHOS	NDUFS1	NADH-ubiquinone oxidoreductase 75-kDa subunit, mitochondrial precursor	79516.3	5.89											↓	

(continued)

Table 1. (Continued)

Energy metabolism process	*Gene name*	*Protein name*	*MW (th)*	*pI (th)*	*BA10 2DE*	*BA9 2DE-DIGE*	*BA24 2DE*	*BA24 2DE**	*CC 2DE*	*BA9 2DE*	*IC 2 DE-DIGE*	*BA46 2DE*	*BA38 Shotgun*	*BA46 Shotgun*	*BA22 2DE*	*Thalamus shotgun 2DE*
OXPHOS	NDUFS3	NADH-ubiquinone oxidoreductase 30 kDa	30241.5	6.98									↓			
OXPHOS	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2	27349.5	8.22									↓	↑		
Oxidative stress	PRDX1	Peroxiredoxin 1	22110.4	8.27		↓										
Oxidative stress	PRDX2	Peroxiredoxin 2	21891.9	5.66		↓								↑		
Oxidative stress	PRDX6	Peroxiredoxin 6	25035	6.00										↑	↑	
Oxidative stress	GSTM3	Glutathione-S-transferase M3 (brain)	26559.6	5.37		↓										
Oxidative Stress	GSTTLp28	Glutathione transferase omega	27565.9	6.24		↓										
Oxidative stress	GSTP1	Glutathione S-transferase P	23355.8	5.43												↓
Oxidative stress	BR1	Carbonyl reductase 1	30374.9	8.55		↓								↓		↑
Oxidative Stress	CBR3	Carbonyl reductase 3	30850.3	5.82		↓										
Oxidative stress	QDPR	Quinoid dihydropteridine reductase	25789.5	6.90		↓									↑	↑
Penthose phosphate pathway	TKT	Transketolase	67877.6	7.58								↑				↑
Oligodendrocyte metabolism	CNP	2′,3′-cyclic nucleotide 3′ phosphodiesterase	47578.6	9.17		↓							↓	↓		
Catalytic activity in energy pathways	CA2	Carbonic anhydrase 2	29114.9	6.86	↓		↑						↓			
Catalytic activity in energy pathways	CKB	Creatine kinase, B chain	42644.7	5.34		↓	↑	↓	↓					↑	↑	
Transaminase activity in energy pathways	GLUL	Glutamate-ammonia ligase (glutamine synthase)	42064.4	6.43		↓										↑
Transport	TF	Transferrin	79310.0	6.97		↓	↓			↓		↓				
Transport	ATP6V1A	Vacuolar ATP synthase catalytic subunit A	68178.3	5.16						↓		↑		↑	↑	

MW, molecular weight; pI, isoelectric point; th, theoretic; 2DE, two-dimensional gel electrophoresis; 2DE-DIGE, fluorescent two-dimensional difference gel electrophoresis; CC, corpus callosum; IC, insulate cortex; BA, Brodmann area; BA9, frontal cortex; BA10, frontal cortex (frontopolar region); BA22p, posterior superior temporal gyrus (Wernicke area); BA24, anterior cingulated cortex; BA46, dorsolateral prefrontal cortex; BA38, anterior temporal lobe. References from left to right (48, 72, 13, 4, 78, 70, 71, 61, 62, 63, 64, and 65).

hippocampus (33). Moreover, decreased levels of arachidonic acid (AA) in brain tissue and red blood cells have been observed in SZ patients (89, 91). Considering that ROS are a natural product of AA metabolism, any impairment of AA processing may lead to an imbalance in ROS production. This would, in turn, lead to oxidative-stress responses, which would have a number of detrimental consequences to mRNA, protein, and lipid pathways in cells.

One postulate on the pathophysiology of SZ suggests that the disorder may be caused by perturbations in prostaglandin pathways (41). The impaired lipid metabolism observed in SZ brain tissues has led researchers to explore peripheral tissues not only to comprehend better their role in the pathogenesis, but also to identify potential biomarkers. Recently, the levels of free fatty acids such as phosphatidylcholine and ceramides have been found to be altered in the SZ PFC and in peripheral RBCs (77).

Accumulation of ceramides may lead to insulin resistance, neurodegeneration, and oxidative stress (reviewed in 16). Isoprostenes are prostaglandin isomers produced from arachidonic acid that may be useful as an indicator of free radical generation or oxidative stress. These molecules have been found at increased levels in the urine of SZ second-generation antipsychotic-treated patients (18). Phosphatidylethanolamine (PE), a membrane structural molecule that provides membrane fluidity, was found decreased in the caudate region of SZ brain tissue (89). Abnormal distribution of PE was proposed as explanatory mechanism for the overall PE decrease in membrane of patients with schizophrenia (69).

Proteome studies have also led to an increased understanding of the impaired lipid-metabolism pathways in SZ. PRDX6, which plays a role in cellular phospholipid turnover by acting as a Ca^{2+}-independent PLA2, and palmitoyl protein thioesterase 1 (PPT1), a key enzyme for fatty acid synthesis, have been shown to be increased in the DLPFC and WA from SZ patients (63, 64). Moreover, decreased expression of apolipoprotein A1 (APOA1) has been found in the cerebrospinal fluid, serum, RBCs, and liver (44, 54, 73), and increased expression of ApoE has been found in SZ DLPFC (63). At the transcript level, mRNAs encoding a number of fatty acid β-oxidation enzymes, such as peroxisomal acyl-CoA oxidase and short- and long-chain dehydrogenases, have been found to be increased. Moreover, several transcripts involved in lipid-biosynthesis pathways were decreased (72).

Antipsychotic treatment may have an influence on cellular lipid composition and therefore on the differential expression of lipid metabolism–related proteins. Experiments in Chinese hamster ovary-7 (CHO-7) cells have shown that the addition of antipsychotics led to increased lipogenic gene expression and disrupted intracellular trafficking of lipoprotein-derived cholesterol (52). Moreover, APOA1 was significantly increased in rats treated with chlorpromazine (54). Although clozapine is efficacious in the treatment of SZ, side effects such as body-weight gain and elevated cholesterol levels are common. The clozapine-induced impairments in lipid and glucose metabolism could also lead to increased ROS and differential activity of antioxidative enzymes (22). This possibility has been supported in studies that found higher levels of triglycerides, total cholesterol, and phospholipids, along with increased SOD1 and lower selenium-dependent GPx activities in SZ patients receiving prolonged treatment with clozapine (67). These data support the case for complex and systemic alterations of lipid, oxidative stress, glucose metabolism, and insulin signaling in SZ pathophysiology and in the mechanism of antipsychotic treatments.

Other energy pathways

Proteomic studies of the PFC and thalamus from SZ patients have identified differential expression of transketolase (TKT), an enzyme of the pentose phosphate pathway (PPP). The main function of the PPP is the generation of redox potential through the production of NADPH, which is essential for the oxidative phosphorylation process. In addition, NADPH plays a role in cell defense against ROS. It is interesting in this case that we also found that NADPH rates were altered in SZ thalamus (65).

Vacuolar ATP synthase catalytic subunit A (ATP6V1A) also was found to be differentially expressed in the PFC and ATL of SZ patients. ATP6V1A is a subunit of V-ATPase, an enzymatic complex that mediates acidification of eukaryotic intracellular organelles. Acidification is necessary for correct functioning of synaptic vesicles, and compromised function of the V-ATPase may therefore lead to impaired neurotransmission.

Glutamine synthase (GLUL) was found to be differentially expressed in the PFC and thalamus of SZ patients. This enzyme catalyzes the synthesis of glutamine, which plays a role in cell proliferation, inhibition of apoptosis, and cell signaling.

Other proteins that are involved in the generation of cellular energy and oxidative response, such as carbonic anhydrase 2 (CA2), creatine kinase (CKB), transferrin (TF), and 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNP) were consistently found to be differentially expressed in SZ brain tissue, as presented in Table 1.

Potential confounding factors

The data presented in this review were obtained by using human samples. Most of the studies cited used samples from long-term antipsychotic–treated patients. Factors such as postmortem interval and sample pH are especially important to take into account when energy metabolism and antioxidant enzymes are discussed, as such factors can alter the proteome. However, several proteins have been found to be altered consistently by different research groups in distinct sets of samples, derived from patients with different treatment regimens and lifestyles. This supports the case that many of these effects are disease associated. Studies carried out in peripheral tissues from first-onset, drug-naïve SZ patients (29, 39, 73, 77) have corroborated these findings. In addition, some of the gene products described here as being involved in the pathophysiology of SZ have also been implicated by other studies that used alternative technologies, such as genomic and transcriptomic approaches, providing additional support for the findings.

One important question that should be considered is whether mitochondrial dysfunction and lipid oxidation are unique to SZ. Mitochondrial failure and oxidative stress as well as dysregulation of glycolytic enzymes have also been proposed as causative factors in Alzheimer disease (1, 10). Disagreement is found in the literature about the extent to which SZ and bipolar disorder manifest dysregulation of energy-metabolism proteins (34, 70, 72). This disagreement may be explained in part by different methods of analysis and by the fact that no method provides complete coverage of the

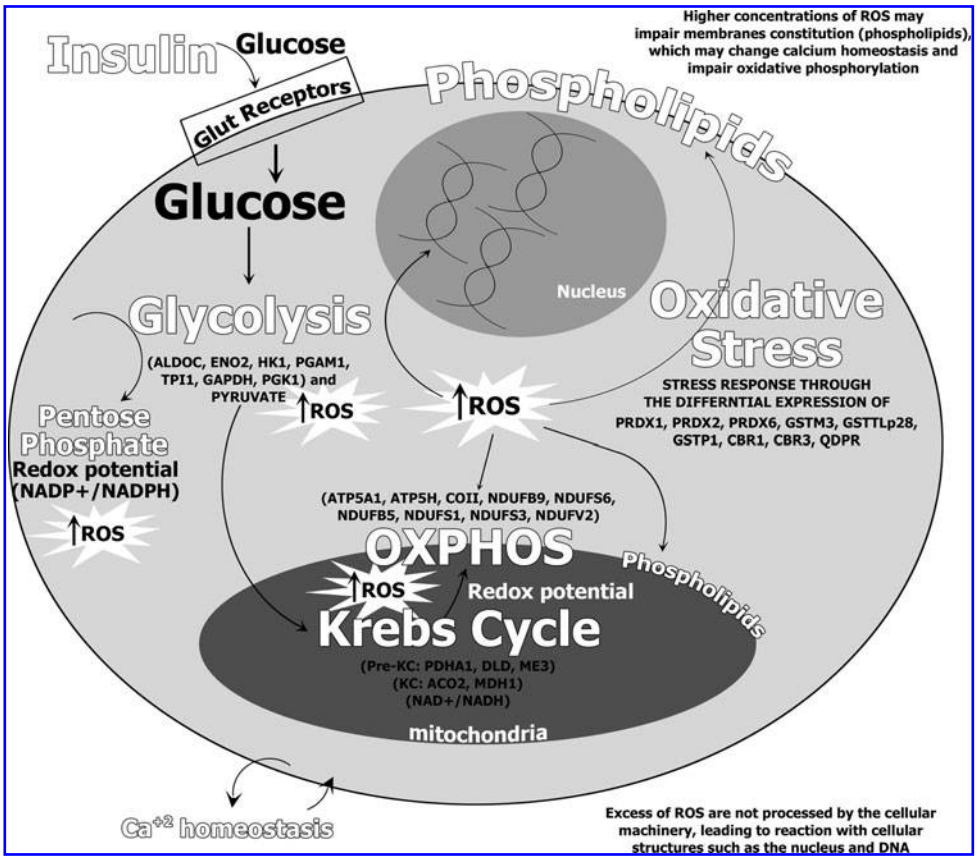

FIG. 2. Summary of biochemical processes involved in schizophrenia and the central role of reactive oxygen species. ROS, reactive oxygen species.

proteome. However, most studies agree that at least some perturbation of metabolic pathways occurs in SZ.

Conclusions and Perspectives

The most consistent pathway that seems to be impaired in SZ brain and periphery appears to be glycolysis. Because this is the pathway in which energy production starts from glucose processing, it is expected that other components of the energy-production pathway will also be compromised (see diagram, Fig. 2). Effects on glycolysis could also lead to perturbations of the Krebs cycle, especially regarding acetyl-CoA and oxaloacetate production from pyruvate, which has been shown to be dysregulated in SZ (65). In turn, alterations in Krebs-cycle enzymes could also lead to impairments in OXPHOS, which is also dysregulated in SZ (reviewed in 66). These three key processes—glycolysis, the Krebs cycle, and OXPHOS—also are responsible for the metabolism and production of redox potential through the generation of NAD/NADH, NADP/NADPH, and FAD/FADH. Perturbations of this pathway could lead to generation of ROS, which would lead to damaged biologic membranes and DNA. An imbalance of ROS, which probably cannot be properly neutralized (92), establishes an oxidative-stress state, which is also borne out by the proteomic evidence, and also may perturb Ca^{2+} buffering, which is involved in regulating important pathways, such as lipid homeostasis and hormonal signaling. Proteomic data suggest that the fine tuning required by the enzymatic cellular machinery for energy production appears to be imbalanced in SZ, leading to increased ROS production and an oxidative-stress state.

The proteome studies presented (Table 1) have revealed differential expression of concordant proteins in SZ, but, in some cases, protein expression shows different patterns and often counterregulation with respect to brain region. Such a finding is expected and has been observed previously, because of tissue-specific differences in protein expression and because some brain regions may be counterregulated, depending on whether they are connected via excitatory or inhibitory interneurons.

It is also important to consider some variables, specifically, the effects of antipsychotic treatment on the proteome, because many of the samples under study came from long-term–treated SZ patients. The altered metabolism/oxidative stress-related proteins in SZ not only serve as potential biomarkers, but have also contributed to the further understanding of SZ pathobiology (60), which is required for more-effective treatments and prognosis. For example, the impairment of glucose metabolism, added to previous results of insulin resistance in SZ and antipsychotic treatment, have led researchers to investigate the potential role of antidiabetic drugs as a treatment enhancer. The coadminstration of the antipsychotic clozapine and the antidiabetic metformin have improved the metabolic control in SZ patients, decreasing body weight and insulin and triglycerides levels, without mental-state impairment (11). Similarly, the co-treatment with olanzapine and metformin regulates body weight and controls carbohydrate metabolism, without compromising antipsychotic efficacy (3).

We believe that the differentially expressed proteins, as well as the impaired biochemical pathways revealed by proteome studies of SZ brain and peripheral tissues discussed here, may be potential targets for further studies.

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Abbreviations Used

2D-DIGE = fluorescent 2-DE difference gel electrophoresis
2DE = two-dimensional gel electrophoresis
AA = arachidonic acid
ACC = anterior cingulate cortex
ACO2 = aconitase 2
ALDOA = aldolase A
ALDOC = aldolase C
APOA1 = apolipoprotein A1
ApoE = apolipoprotein E
ATL = anterior temporal lobe
ATP = adenosine triphosphate
ATP6V1A = vacuolar ATP synthase catalytic subunit A
Ca^{2+} = calcium
CA2 = carbonic anhydrase 2
CBR1 = carbonyl reductase 1
CBR3 = carbonyl reductase 3
CHO-7 = Chinese hamster ovary-7
CKB = creatine kinase
CNP = 2′,3′-cyclic nucleotide 3′ phosphodiesterase
CNS = central nervous system
CS = citrate synthase
DLPFC = dorsolateral prefrontal cortex
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
GLUL = glutamine synthase
GPx = glutathione peroxidase
GR = glutathione reductase
GST = glutathione transferase
ICAT = isotope-coded affinity tag
ICPL = isotope-coded protein labeling
iTRAQ = isobaric tag for relative and absolute quantitation
LC-MS/MS = liquid chromatography–tandem mass spectrometry
MDH1 = malate dehydrogenase 1
MDH2 = malate dehydrogenase 2
MS = mass spectrometry
OXPHOS = oxidative phosphorylation
PCP = phencyclidine
PDHA1 = pyruvate dehydrogenase E1 component alpha 1
PE = phosphatidylethanolamine
PET = positron emission tomography
PFC = prefrontal cortex
PGAM1 = phosphoglycerate mutase 1
PLA2 = phospholipase A2
PPP = pentose phosphate pathway
PPT1 = palmitoyl protein thioesterase 1
PRDX = peroxiredoxin
QDPR = quinoid dihydropteridine reductase
RBCs = red blood cells
ROS = reactive oxygen species
SOD1 = superoxide dismutase [Cu-Zn]
SZ = schizophrenia
TF = transferrin
TKT = transketolase
WA = Wernicke area
WB = Western blot

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