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Research Report

Paliperidone as a mood stabilizer: A pre-frontal cortex synaptoneurosomal proteomics comparison with lithium and valproic acid after chronic treatment reveals similarities in protein expression

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ARTICLE INFO

Article history:

Accepted 1 July 2008

Available online 15 July 2008

Keywords:

Mood stabilizers

Paliperidone

Lithium

Valproate

Proteomics

Synaptoneurosomes

ABSTRACT

A series of recent studies has demonstrated that the molecules involved in regulation of neuronal plasticity are also involved in the mode of action of antidepressants and mood stabilizer drugs. Intracellular calcium signaling, energy metabolism, and neuronal plasticity can be influenced by inducing axonal remodeling and increasing levels of certain synaptic proteins. Because antipsychotic drugs are used as mood stabilizers our studies focused on a newly-marketed antipsychotic drug, paliperidone. We determined changes in rat synaptoneurosomal proteins after chronic treatment with paliperidone, lithium salt, or valproic acid in order to find similarities or differences between the mode of action of paliperidone and these two classical mood stabilizers. We determined differential protein expression profiles in prefrontal cortex (PFC) of male Sprague–Dawley rats ($n=4$ /group). Synaptoneurosomal-enriched preparations were obtained from PFC after chronic treatment with these three drugs. Proteins were separated by 2D-DIGE and identified by nano-LC-MS/MS. We observed similar protein expression profiles at the synaptoneurosomal level, suggesting that the mode of action for paliperidone is similar to that of lithium and valproic acid. However, the expression profile for paliperidone was more similar to that of lithium. Pathways affected in common by these two drugs included oxidative phosphorylation, electron transport, carbohydrate metabolism, and post-synaptic cytokinesis implicating the effects of these drugs in signaling pathways, energy metabolism, and synaptic plasticity.

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Abbreviations: HC, Hierarchical clustering; SVM, support vector machines; POI, proteins of interest; FT, Fourier transformed

1. Introduction

Paliperidone was recently approved by the United States Food and Drug Administration (FDA) with an indication for the treatment of schizophrenia. Although it is the 9-hydroxy, active metabolite of risperidone, and shares some similarities in its receptor binding profile with that of risperidone (Richelson and Souder, 2000), there are suggestions that paliperidone is pharmacologically distinct from its parent compound (Dremencov et al., 2007). Indeed, animal data suggest that paliperidone may differentially affect serotonin and norepinephrine neuronal firing compared to risperidone, when used in combination with serotonin-selective re-uptake inhibitor (SSRI) antidepressant (Dremencov et al., 2007). In addition, based on animal studies, it has been suggested that paliperidone may be especially useful in the therapy of depression in those patients resistant to SSRI's (Dremencov et al., 2007). We hypothesize that paliperidone could be used as a mood disorder stabilizer in patients with bipolar disorder.

Also called manic-depressive illness, bipolar disorder is characterized over a lifetime by recurrent episodes of depression and mania, which is considered at the opposite pole from depression in the mood spectrum (hence, the term “bipolar”). The amplitude and the frequency of the cycling in bipolar disorder can be attenuated or eliminated by a class of drugs called mood stabilizers.

Lithium ion (Li⁺) in the form of various salts (e.g., chloride or carbonate; referred to here as “lithium”) is the first established mood stabilizer, although it was first used as an antimanic drug in the 1940 s. Drugs on the anticonvulsant class, such as valproic acid (“valproate”) are now commonly used to treat bipolar disorder. Valproate is the only drug of its class approved by the FDA for use as an antimanic drug, but recent data support its use as a mood stabilizer.

The majority of studies to date on antimanic and mood-stabilizing drugs are based on genomic techniques that lack the specificity possible by proteomic techniques. It is well known that microarray and genomic studies have limitations in terms of the information that can be obtained after drug treatment. For example, changes in levels of mRNA with drug treatment do not necessarily reflect changes in protein levels, which can be provided by proteomics studies. The majority of proteomic studies to date focus on specific brain regions (e.g., prefrontal cortex) and not on specific processes within a region (e.g., the synapse). In order to understand changes in synaptic transmission induced by drugs used to treat mood disorders, it is necessary to narrow the scope of proteomic studies and focus on the synaptoneurosome.

Synaptoneurosomal preparations are highly enriched in pinched-off, resealed pre-synaptic processes (synaptosome) attached to resealed post-synaptic processes (neurosome). These preparations retain normal functions of neurotransmitter release, receptor activation, and various post-synaptic responses including signaling pathways and protein synthesis (Greenough et al., 2001). Therefore, Synaptoneurosomal-enriched protein preparations include a collection of pre- and post-synaptic proteins involved in a variety of pathways.

The objective of this study was to obtain data to support our hypothesis that paliperidone can be used clinically as a

mood stabilizer drug. In order to accomplish this goal, we determined changes in expression in synaptoneurosomal-enriched pre-frontal cortex (PFC) preparations of rat brain after chronic treatment with paliperidone, lithium, and valproate. In keeping with the clinical data on the latter two drugs and in support of our hypothesis, paliperidone showed changes more like lithium than that of valproate.

2. Results

2.1. Synaptoneurosomal preparations and Western blots

We were able to isolate synaptoneurosomal proteins from the PFC of rat brain using the protocol for extraction of synaptoneurosomal-enriched preparations described by Hollingsworth, Villasana and collaborators (Hollingsworth et al., 1985; Villasana et al., 2006). According to protein quantitation, we were able to obtain an average 763.18 µg of total synaptoneurosomal-enriched proteins from each PFC using this protocol. As reported by Villasana and collaborators, we found this method reliable and consistent. To analyze further our preparations, we performed Western blots and used glial-, post-, and pan-synaptic protein markers. Our results are shown in Fig. 1. Consistent with the results reported by Villasana, we observed a decrease of GFAP-50 and an increase in PSD-95 and CaMKII-60 proteins, results indicating that our synaptoneurosomal pellets were enriched in both pre- and post-synaptic proteins.

2.2. Hierarchical clustering analysis

Although the number of spots present in each gel varied from 1966 to 2341, analysis of spots significantly ($P < 0.05$) represented in all animals from the same group revealed an average of 2183 spots in the lithium-, valproic acid-, and saline-treated groups. The tartaric acid and paliperidone groups shared an average of 2084 spots per group. Cluster analysis of paliperidone, lithium, and valproic acid was performed with and without the vehicle group (tartaric acid) or the saline control group. A comparison of the three drugs alone revealed that paliperidone and lithium treatment resulted in similar protein expression profiles and clustered together as seen in Fig. 2.

2.3. Spot identification

We observed common spots when comparing the lithium and paliperidone treatment groups, results suggesting

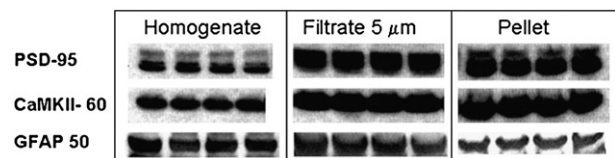


Fig. 1 – Enrichment of four synaptoneurosomal preparations (four different animals). GFAP-50 (50kD) (glial marker) decreased as the isolation progressed. PSD-95 (95 kD) and CaMKII-60 (60 kD) were increased in the final pellet showing enrichment in synaptoneurosomal proteins in these preparations.

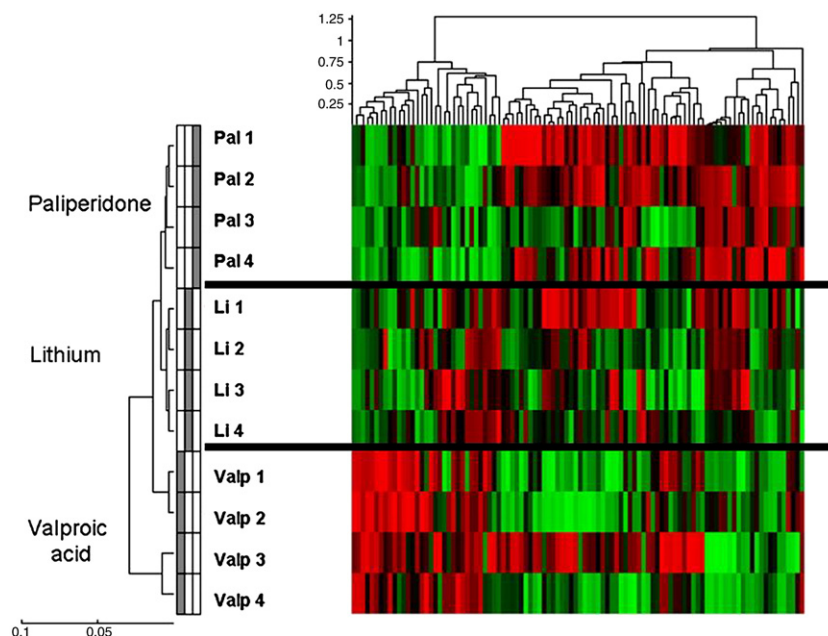


Fig. 2 – Hierarchical clustering of proteins obtained after analysis of 12 gel images (four biological replicates per treatment). Each row is representative of PFC synaptoneurosomal proteins from one animal. Each column represents an individual protein. Red indicates increased expression (upregulation) and green indicates a decrease (downregulation). Numbers indicate individual animals (1, 2, 3 and 4).

similarities in changes of expression elicited by treatment with these two drugs. We only observed one spot common to both lithium and valproic acid (spot ID 6185), but it was not significantly different from the saline control (P -value=0.609). Therefore, we did not identify it by MS. We found two spots common to both valproic acid and paliperidone (6099 and 6055) and 9 spots common to lithium and paliperidone. Some spots were found present in the lithium and valproic acid groups and not in the paliperidone group, results suggesting differences between the latter and these two drugs.

Thirteen spots defined the lithium and valproic acid groups while 16 defined the paliperidone group according to SVM. Significant differences were seen in the lithium, valproic acid, and paliperidone groups when compared to the saline control. The tartaric acid vehicle group was also compared to the saline control and the other group treatments, but no significant differences or similarities were found.

Spot identification number and location for those proteins represented in all biological replicates from a group, with 2 fold or more change in expression with respect to the saline control, 200 units or more volume and P -values < 0.05 are shown in Fig. 3.

2.4. Changes in expression common to all three drug treatments compared to saline

One spot, 7515, was found significantly up regulated by more than 2 fold in all drug treatments.

2.4.1. Valproic acid-treatment group

13–16 proteins defined each treatment group. We found 3 spots: 6049, 5757, and 7215 significantly up-regulated in the valproic acid group in comparison to the lithium and paliperidone

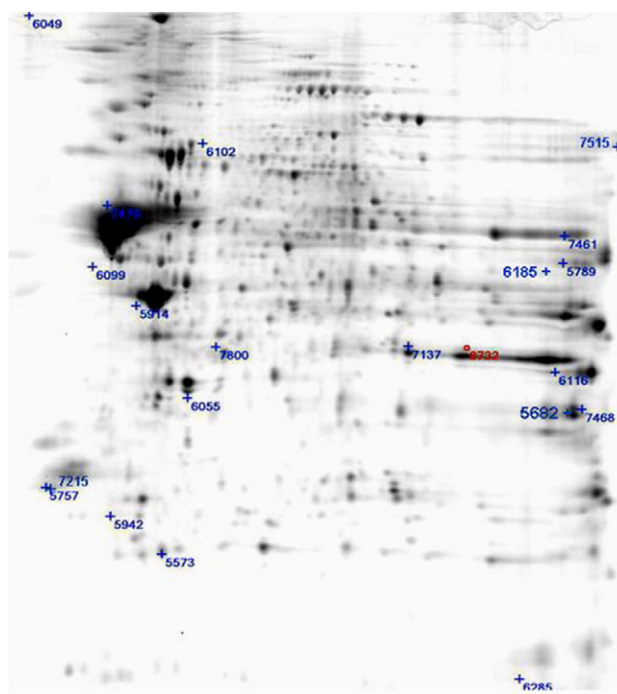


Fig. 3 – Representative image of a 2D-DIGE gel showing spot identification numbers for proteins found differentially expressed after lithium, valproic acid and paliperidone chronic treatment in synaptoneurosomal enriched preparations of rat PFC tissue. Spots showing significant changes in mean normalized volume after drug treatment were selected for analysis and are indicated by crosshairs. Spot 8732 was faint in this particular gel (lithium treated animal) therefore is indicated in red.

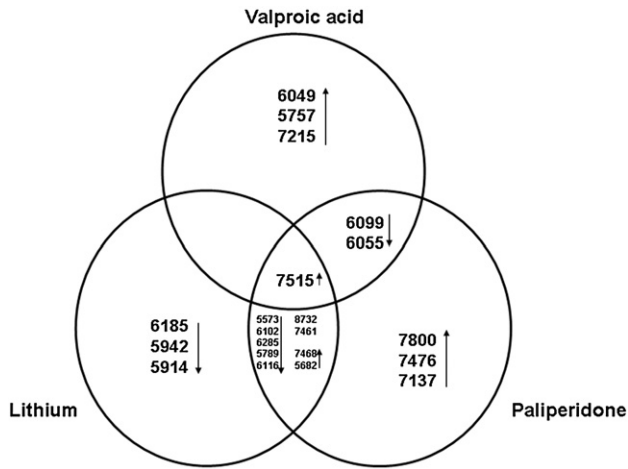


Fig. 4 – Spot ID numbers of proteins that exhibited differential expression in each group treatment compared to the saline control and to each other. Arrows indicate expression as follows: upregulated (↑) and downregulated (↓) compared to the saline control.

groups. Spot 6099 was down-regulated in saline, unchanged with respect to lithium, and up-regulated in the paliperidone group. Spot 6055 was down-regulated in the valproic acid and paliperidone groups and unchanged in the lithium group.

2.4.2. Lithium-treatment group

We found 3 spots (5942, 6185 and 5914) significantly down-regulated with lithium treatment compared to treatment with saline, paliperidone, or valproic acid.

2.4.3. Paliperidone-treatment group

We found 3 different spots (7800, 7476 and 7137) up-regulated in paliperidone compared to those for the other groups.

2.5. Lithium- and paliperidone-treatment groups

Since treatment with lithium or paliperidone resulted in similar expression profiles as determined by clustering analysis, a comparison of spots common to these two treatments vs. the valproic acid and saline groups was made. We found 5 spots down-regulated in both lithium and paliperidone groups (5573, 6102, 6285, 5789, and 6116) and two up-regulated (7468 and 5682). Spot 8732 was down-regulated (to undetectable levels) in the lithium group

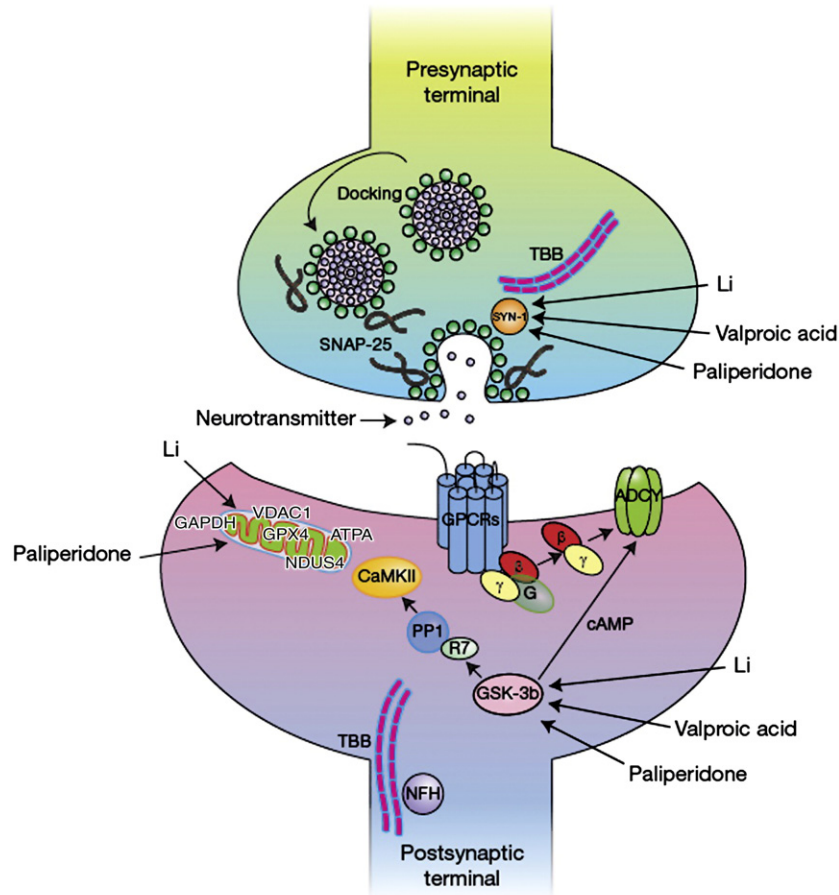


Fig. 5 – Cartoon representing the effects of Lithium, valproic acid and paliperidone in synaptoneurosomal enriched preparations. Lithium and paliperidone appeared to have effects on mitochondrial proteins associated with the electron transport chain. All drugs seemed to have a direct or indirect effect on GSK-3 (post-synaptic) and SYN-1 (pre-synaptic).

Table 1 – Protein identification by MS. Proteins are shown arranged by groups in terms of drug treatment

Protein ID	ID	Accession	Fold vs. saline	P-value	MW (Da)	Score	Protein name
<i>Differentially expressed after valproic acid treatment only</i>							
NFH_RAT	6049	P16884	2.15	4.01E–05	115593	4535	Neurofilament triplet H protein (200 kDa neurofilament protein)
SNP25_CHICK	5757	P60878	2.26	1.86E–07	23528	6274	Synaptosomal-associated protein 25
SNP25_CHICK	7215	P60878	2.33	1.30E–05	23528	5632	Synaptosomal-associated protein 25
<i>Differentially expressed after valproic acid and paliperidone treatment</i>							
PP1R7_RAT	6099	Q5HZV9	SAL=–1.01 PAL=2.62	<1.00E–15	41385	6705	Protein phosphatase 1 regulatory subunit 7
GBB2_HUMAN	6055	P62879	SAL=–1.25 PAL=–0.81	1.20E–15	37917	8954	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta 2 (Transducin beta chain 2)
<i>Differentially expressed after lithium treatment only</i>							
TBB_PIG	5942	P02554	–0.48	3.17E–06	50285	2390	Tubulin beta chain (Beta tubulin)
GPX41_RAT	6185	P36970	–0.5	4.10E–03	22847	555	Phospholipid hydroperoxide glutathione peroxidase, (EC 1.11.1.12) (PHGPx)
GNAO1_CRILO	5914	P59216	–0.35	9.30E–04	40482	9359	Guanine nucleotide-binding protein G(o) subunit alpha 1
<i>Differentially expressed after paliperidone treatment only</i>							
PP1A_BOVIN	7800	Q3T0E7	2.1	7.00E–04	38229	3789	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit (EC 3.1.3.16) (PP-1A)
TBB2C_BOVIN	7476	Q3MHM5	2.34	2.66E–06	50255	8760	Tubulin beta-2C chain
G3P_RAT	7137	P04797	2.44	3.59E–06	35828	531	Glyceraldehyde-3-phosphate Dehydrogenase
<i>Differentially expressed in all treatments</i>							
SYN1_RAT	7515	P09951	Li=2.11, VPA=2.20, PAL=2.17	1.10E–16	74114	8358	Synapsin-1 (Synapsin I)
<i>Differentially expressed in Lithium and paliperidone</i>							
PEBP1_RAT	5573	P31044	Li=–0.42, PAL=–0.42	6.44E–08	20771	5412	Phosphatidylethanolamine-binding protein 1 (PEBP-1) (HCNPPP) (23 kDa morphine-binding protein)
VDAC1_RAT	7468	Q9Z2L0	Li=2.20, PAL=2.3	1.00E–03	30720	8018	Voltage-dependent anion-selective channel protein 1 (VDAC-1) (rVDAC1) (Outer mitochondrial membrane)
VDAC1_RAT	5682	Q9Z2L0	Li=2.01, PAL=2.46	4.60E–03	30720	8018	Voltage-dependent anion-selective channel protein 1 (VDAC-1) (rVDAC1) (Outer mitochondrial membrane)
GRP75_RAT	6102	P48721	Li=–1.77, PAL=undetected	2.40E–02	73858	749	Glucose related polypeptide 75 kD (HSP70)
G3P_RAT	8732	P04797	Li=undetected, PAL=2.0	5.96E–11	35828	604	Glyceraldehyde-3-phosphate dehydrogenase
ATPA_RAT	7461	P15999	Li=1.07, PAL=undetected	1.07E–10	59754	1017	ATP synthase alpha subunit
NDUS4_RAT	6285	Q5XIF3	Li=–0.50, PAL=–0.33	4.10E–03	19741	332	NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial [Precursor]
SEPT7_RAT	5789	Q9WVC0	Li=–0.99, PAL=undetected	1.10E–02	50508	488	Septin 7
ROA2_MOUSE	6116	O88569	Li=–0.94, PAL=undetected	3.10E–03	37403	22	Heterogeneous nuclear ribonucleoproteins A2/B1

compared to that for the saline group, and up-regulated in the paliperidone group. Spot 7461 was up-regulated in the lithium group and down-regulated (undetectable) in the paliperidone group.

Common and different spots and their regulation after each drug treatment are shown in the Venn diagram of Fig. 4. Spots common to more than one treatment are shown in the intersecting segments of the diagram. We did not find proteins common to lithium and valproic acid. The locations of relevant proteins in the synapse are depicted in the cartoon shown in Fig. 5.

Protein identification of the spots picked for MS is shown in Table 1. Proteins are arranged by groups according to their differential expression after a particular drug treatment.

3. Discussion

Our findings correlate with reports by other research groups as the proteins identified in our study are involved in synaptic transmission as well as reception. Furthermore, these proteins have been isolated previously from synaptosomal preparations using sucrose and Ficoll gradients (Ogden et al., 2004; Schrimpf et al., 2005; Witzmann et al., 2005). Our goal was to focus on the protein profile of synaptoneuroosomes isolated from the PFC of the rat brain after treatment with mood stabilizers since dysfunction of the PFC has been linked to the pathophysiology of mood disorders (Baxter et al., 1989;

Blumberg et al., 1999; Quraishi and Frangou, 2002; Rubinsztein and Sahakian, 2002; Blumberg et al., 2003; Haldane and Frangou, 2004; Kronhaus et al., 2006).

We used three different chemical compounds: paliperidone, a ketone used as an atypical antipsychotic drug; lithium chloride, an inorganic salt and prototypical mood-stabilizer; and an organic acid, 2-propylpentanoic acid or valproic acid, originally formulated as an anticonvulsant. These three drugs elicited similar protein expression profiles at the synaptoneurosomal level after chronic treatment. However, the protein expression profile for paliperidone was more similar to that of lithium than to that of valproate, suggesting that paliperidone and lithium share some common modes of action. Our results support the hypothesis that paliperidone has mood stabilizing effects.

In terms of expression, only one protein, identified as 7515 or Synapsin-1 (SYN1) was found up regulated in all treatments compared to the saline control. Low levels of SYN1 have been associated with the cortex of patients with both major depressive disorder and bipolar disorder (Hayden and Nurnberger, 2006). Synapsin 2 (SYN2) has been reported to be down regulated in the rat frontal cortex after exposure to chronic fluoxetine (antidepressant) and GR205171 (a neurokinin receptor 1 (NK1) antagonist) treatment (Carboni et al., 2006). SYN1 and SYN2 are involved in mediating the brain-derived neurotrophic factor (BDNF) mediated modulation of glutamate release (Jovanovic et al., 2000). SYN1 is a neuronal phosphoprotein that coats small synaptic vesicles (SSV), binds the cytoskeleton by interacting with tubulin and spectrin, and is believed to function in the regulation of neurotransmitter release (Bennett et al., 1991). Our findings of SYN1 up-regulation suggest that paliperidone, lithium, and valproic acid share one common protein involved in neurotransmitter release and synaptic plasticity. Clustering of SYN1 in developing neurons has previously been associated with lithium and valproic acid treatment through inhibition of glycogen synthase kinase 3 beta (GSK-3 β), a serine/threonine kinase involved in the insulin and WNT signaling pathways (Klein and Melton, 1996; Ferkey and Kimelman, 2000; Frame and Cohen, 2001; Hall et al., 2002). Valproic acid promotes neurite outgrowth (Illig et al., 2000; O'Leary et al., 2000), increases SYN1 clustering, activates extracellular signal-regulated kinases, increases growth cone size and its associated protein 43 (GAP43) and bcl-2, and affects nerve regeneration (Manji et al., 2000; Hall et al., 2002; Tariot et al., 2002). Through its abilities to inhibit GSK-3 β (Chen et al., 1999), valproic acid may play a role in blocking the synthesis of pro-apoptotic factors that contribute to neuronal loss (Perez et al., 2003). Inhibition of GSK-3 β by lithium mimics the effect of WNT signaling resulting in changes in gene expression and cell fate decisions (Klein and Melton, 1996). More recently, WNT signaling through GSK-3 β has been implicated in neuronal plasticity as WNT and lithium induce changes in the morphology of axons and synaptic protein clustering (Lucas and Salinas, 1997; Lucas et al., 1998; Goold et al., 1999; Hall et al., 2000). It has been proposed that in addition to inhibition of GSK-3 β lithium might induce its N-terminal phosphorylation through a secondary inhibition mechanism that includes inhibition of PP1 the phosphatase that dephosphorylates GSK-3 (Zhang et al., 2003). Together these observations lead to the hypothesis

that paliperidone might have an inhibitory effect on GSK-3 β and the WNT signaling pathway similar to that of lithium and valproic acid. This hypothesis is supported by the observation that antipsychotics, independent of class, have a profound effect on both β -catenin and GSK-3 (phosphorylated and non-phosphorylated) in a region of the brain (PFC) that is innervated by the mesolimbic dopamine (DA) system and believed to play a vital role in both schizophrenia and the action of antipsychotics such as risperidone (Alimohamad et al., 2005).

Proteins whose expression levels changed exclusively after valproic acid treatment in our study were 6049 (NFH), 5757 and 7215, both identified as SNAP-25, 6099 (PP1R7) and 6055 (GBB2). NFH, is a 200 kD protein involved in the maintenance of neuronal caliber. NFH has an important function in mature axons that is not observed by the two smaller NF proteins and its phosphorylation has been implicated in the mechanism of regulation of neurofilament transport (Ackerley et al., 2003). In addition, the phosphorylated form of NFH has been considered an axon marker (Raabe et al., 1996). Our findings in terms of NFH expression indicate that chronic valproic acid treatment increases levels of NFH in synaptoneurosomes. Our observations of the presence of NFH in synaptoneurosomal-enriched preparations in a rodent are consistent with those of Schrimpf's group in 2005. Their proteomics data showed that NFH was detected in synaptosomes isolated from whole mouse brain using Ficoll gradients (Schrimpf et al., 2005). Our results also correlate with those of Dou and collaborators (Dou et al., 2003). Their group reported a decrease in expression of NFH in neurons from the basal ganglia in mice treated with valproic acid after 7, 14 and 21 days suggesting that valproic acid might regulate expression of NFH.

Spots 5757 and 7215 were located close to each other in the gels with a similar molecular weight around 27.8 kD and were identified by MS as SNAP-25, a pre-synaptic protein associated with vesicle docking and fusing (Hodel, 1998). Our results of the presence of SNAP-25 in synaptoneurosomal-enriched preparations support those of Schrimpf and Witzmann in synaptosomes (Schrimpf et al., 2005; Witzmann et al., 2005). The location of these spots suggested slightly different isoelectric points (pI). It is possible that one of the spots is a post-translationally modified, perhaps phosphorylated form of the same protein. We are currently investigating the status of the phosphoproteome in synaptoneurosomal preparations of rat PFC after treatment with valproic acid, lithium and paliperidone with aims to determine phosphorylation changes induced by drug treatment. SNAP-25 is a member of the soluble N-ethylmaleimide-sensitive fusion protein receptor (SNARE) protein complex essential for neurotransmitter release. Although the physiological relevance of SNAP-25 phosphorylation in brain function has not yet been determined phosphorylation of SNAP-25 has been reported as essential for regulation of neuronal function (Nagy et al., 2002; Shoji-Kasai et al., 2002).

Spot 6099 corresponded to PP1R7, a protein implicated in the regulation of a variety of cellular functions such as glycogen metabolism, calcium transport, muscle contraction, intracellular transport, protein synthesis, and cell division (Bollen and Stalmans, 1992). PP1R7 has been associated with mitosis (Stone et al., 1993). PP1R7 is the product of the ppp1r7 or sds22 gene and it has been localized in the nucleus and cytoplasm in yeast (Renouf et al., 1995). Although we are not

aware of any reports documenting the presence of PP1R7 in synaptoneuroosomes, the presence of PP1 in synaptic junctions has been linked to dephosphorylation of Ca_2^+ /calmodulin-dependent protein kinase II (CaMKII) and synapse-enriched phosphoproteins (Shields et al., 1985). It has been proposed that autophosphorylation of CaMKII by itself shows a steeper Ca_2^+ dependence when autophosphorylation is balanced by the dephosphorylation activity of PP1 (Bradshaw et al., 2003). It is possible that our findings are related with regulation of CaMKII phosphorylation since we observed a down-regulation of the regulatory subunit of PP1 in response to valproic acid treatment and up-regulation in response to paliperidone treatment. Further studies are needed to confirm this hypothesis.

Spot 6055 was identified as GBB2, also called transducin beta chain 2 or G protein subunit beta-2. GBB2 is a membrane-associated protein that mediates the effects of numerous G protein-coupled receptors (GPCRs). Receptors belonging to the GPCR family include muscarinic acetylcholine (ACh), DA, beta-adrenergic, 5-HT, metabotropic glutamate, GABA_B, histamine, cannabinoid and neuropeptide receptors. It is unknown if GBB2 is an exclusively pre or post-synaptic protein but our findings in synaptoneurosomal-enriched preparations corroborate the results of Witzmann et al. in rat cerebral cortical synaptosomes (Witzmann et al., 2005). GBB2 was found down regulated after valproic acid and paliperidone treatment. The implications are varied and warrant further study in particular on the effects of this regulation in the cAMP/adenylate cyclase (cAMP/AC) pathway. The beta subunit of G-proteins is essential for receptor recognition. It has been proposed that this subunit acts as a mediator of hormonal inhibition of AC and activation of phospholipase A. Since the effect of valproic acid on AC and cAMP has been well documented, our findings provide further insights into the regulatory mechanism of this pathway. Long-term administration of valproate has been shown to produce a reduction in the density of beta-adrenergic receptors in the brain and it has been suggested that chronic valproate administration also exerts effects at the beta-adrenergic-G-protein (Gs) interaction or at post-receptor sites such as Gs or AC (Chen et al., 1999).

Proteins differentially expressed after lithium treatment included 5942 (tubulin beta chain, TBB), 6185 (Phospholipid hydroperoxide glutathione peroxidase, mitochondrial precursor, GPX41) and 5914 (guanine nucleotide-binding protein G(o) subunit alpha 1 (GNAO1). Our observations of the presence of TBB and GNAO1 in synaptoneurosomal-enriched preparations support the results of Witzmann et al. (2005). TBB, GPX4 and GNAO1 or GAO1, sometimes referred to as GOA-1 or G(o) α were found to be down regulated after chronic lithium treatment. The implications of the lower levels of TBB and GNAO1 might be related to the WNT signaling pathway. TBB and GAO1 have been implicated in the WNT signaling pathway through GSK-3 β regulation. WNT increases axonal spreading and branching in cultured granule cells and it has been related to increases in the levels of SYN1. Lithium mimics WNT-7a in granule cells by inhibiting GSK-3. These results suggest a direct effect of WNT-7a in the regulation of neuronal cytoskeleton and SYN1 in granule cell neurons. It has been proposed that WNT proteins have a novel function in the formation of neuronal connections (Lucas and Salinas, 1997). TBB has been reported to be up

regulated in the hippocampus and down regulated in the frontal cortex of rats after chronic DMP696 treatment (Carboni et al., 2006).

GPX4 also called PHGPX, MCSP; PHGPx; snGPx; snPHGPx, is an enzyme involved in cell detoxification from lipid peroxide-mediated damage through lipid peroxide reduction and it is the only known intracellular antioxidant enzyme that can directly reduce peroxidized phospholipids and cholesterol in membranes (Arthur et al., 1993; Thomas et al., 1990; Yagi et al., 1996). Its exact localization in synaptoneuroosomes has not been determined although it has been associated with mitochondria (Bai and Cederbaum, 2000). The implications of the low levels of GPX4 in synaptoneurosomal preparations in response to lithium treatment are currently unknown.

Chronic paliperidone treatment resulted in differential expression of three distinct spots at the 2 fold or more level: 7800 (serine/threonine-protein phosphatase PP1- alpha catalytic subunit, PP1A) and 7476 (tubulin beta 2C-chain, TBB2C) and 7137 (glyceraldehyde 3-phosphate dehydrogenase). All these proteins were up regulated. The implications of the up-regulation of these proteins in the mechanism of action of paliperidone and its comparison with the effects of Lithium and valproic acid might be related to the mechanism of regulation of GSK-3. It has been recently demonstrated that inactivation of PP1 leads to regulation of GSK-3 (Morfini et al., 2004) suggesting a link between PP1 and GSK-3. Our results provide support to the hypothesis that atypical antipsychotics, in addition to being mood-stabilizers and possible augmenters of antidepressants, can inhibit the activity of GSK3. It has been reported that acute treatment of mice with risperidone rapidly increases the level of brain phospho-Ser-GSK3 in the cortex, hippocampus, striatum, and cerebellum in a dose-dependent manner. It was suggested that these findings may support the pharmacological mechanisms of atypical antipsychotics in the treatment of mood disorders (Li et al., 2007). Our findings of the presence of G3P (spot 7137) correlate with those of Li and collaborators (Li et al., 2004). This group reported the presence of several enzymes from the glycolytic pathway in the post-synaptic density fraction isolated from rat forebrain (Li et al., 2004). This is in agreement with a previous study, which demonstrated by immunoelectron microscopy the presence of G3P in the dendritic spine and post synaptic density. It was postulated that these proteins provide immediate availability of glycolytic source of ATP to the synapse. This is especially important during acute increase in synaptic activity when the mitochondrial supply of energy does not meet the transient and highly localized increased demand in energy (Wu et al., 1997).

Proteins found common to treatment with lithium and paliperidone included 5573 (phosphatidylethanolamine-binding protein 1 (PEBP-1), 7468 (voltage-dependent anion-selective channel protein 1 (VDAC1), 6102 (75kD glucose-regulated protein, GRP75), 8732 (glyceraldehyde 3-phosphate dehydrogenase, G3P), 7461 (ATP synthase subunit alpha, mitochondrial precursor, ATPA), 6285 (NADH dehydrogenase (ubiquinone) iron-sulfur protein 4, mitochondrial precursor, NDUS4), 5789 (Septin 7 or SEPT7) and 6116 (heterogeneous nuclear ribonucleoprotein A2/B1, ROA2). PEBP is the precursor of the hippocampal cholinergic neurostimulating peptide (HCNP), an 11 amino acid peptide that possesses cholinergic

neuronal stimulatory activity. The HCNP is suggested to be able to act independently and also synergistically with nerve growth factor to enhance the production of choline acetyltransferase, which assists in cholinergic development of the medial septal nuclei of the brain (Butterfield et al., 2006). GRP75 also known as heat shock protein 70 or HSP70 was also found altered in the synaptoneurosomal preparations in response to chronic lithium and paliperidone treatment. SEPT7, or CDC10 protein homolog was also affected by both lithium and paliperidone. Its localization in synaptosomes has been previously reported (Witzmann et al., 2005) and it has been linked to cytokinesis (Longtine et al., 1996). ROA2 or hnRNP A2/hnRNP B1, has been implicated in pre-mRNA processing. Much of the regulation of the pathway of gene expression in higher eukaryotic cells is posttranscriptional and involves pre-mRNA processing, the nucleocytoplasmic transport of mRNA, and the translation, stability, and localization of mRNA in the cytoplasm. The A/B group of hnRNP proteins appears to play important roles in the biogenesis and transport of mRNA (Kamma et al., 1999). The implications of PEBP, GRP75, SEPT7 and ROA2 regulation by chronic treatment with mood disorder drugs in the synapse are currently unknown.

Our findings indicate that chronic treatment with both lithium and paliperidone share similar proteins involved in energy production in the mitochondria (G3P, ATPA, NDUS4 and VDAC1). These proteins are involved in glycolysis (G3P) and the electron transport chain (NDUS4 and ATPA). Differential expression of ATPA in the frontal cortex has been reported after chronic DMP696 (a potent corticotrophin-releasing factor receptor 1 (CRFR1) antagonist) treatment (Carboni et al., 2006).

It was unusual to find levels of G3P affected by treatment with lithium and paliperidone since G3P is considered a house keeping gene whose expression remains unmodified under a multitude of conditions. Increases in expression of G3P protein and mRNA have been reported previously after chronic treatment with DMP696 (Carboni et al., 2006) and other antidepressants (Tohda et al., 1999 and Drigues et al., 2003).

Abnormal regulation of nuclear genes coding for mitochondrial proteins has been demonstrated in bipolar disorder. Some of these genes included NDUS, ATPA and VDAC1 (Konradi et al., 2004). NDUS4 is part of Complex I of the electron transport chain implicated in several neurological diseases including bipolar disorder (Karry et al., 2004). Altered neuronal complex I and V activity and consequently energy metabolism may result in abnormal neurotransmission and therefore, abnormal synaptic plasticity and connectivity, leading to the abnormal behavioral symptoms observed in bipolar disorder. It has been suggested that VDAC members play a key role in the regulation of mitochondrial metabolism (Lemasters and Holmuhamedov, 2006).

In summary, our results suggest that similar to lithium and valproate, paliperidone might regulate GSK3 activity. Although the mechanism of regulation of these three drugs appears to involve different pathways, paliperidone induced changes in expression that resembled those observed with lithium and valproic acid at the synaptic level supporting its use as a mood stabilizer. However, paliperidone was more like lithium than like valproate. Pathways affected in common by

treatment with lithium and paliperidone included oxidative phosphorylation, electron transport, carbohydrate metabolism, and post-synaptic cytokinesis implicating the effects of these drugs in signaling pathways, energy metabolism, and synaptic plasticity.

4. Experimental procedures

Unless otherwise noted, chemicals were obtained from Sigma (St. Louis, MO, USA).

4.1. Animal treatment

Four male Sprague–Dawley rats (300–400 g) were used per group treatment. The animals were housed in a temperature- and light-controlled room with free access to food and water. In addition, rats receiving lithium chloride were provided with a bottle of 0.9% saline to minimize the electrochemical imbalance caused by the diuretic properties of lithium. All tests were performed during the first half of a 12 h light/dark cycle following approved procedures by the Mayo Foundation Institutional Animal Use and Care Committee. Animals in each treated group were injected intraperitoneally (i.p.) daily for 28 days with 200 μ l each of the following: lithium chloride dissolved in 0.9% saline (22 mg/Kg), valproic acid in saline (200 mg/Kg) and paliperidone (1 mg/Kg) in 0.3% D,L-tartaric acid dissolved in saline (pH 4.0 adjusted with 1 M NaOH). Two groups were simultaneously injected with equal volumes of vehicle as controls: 0.9% saline and 0.3% tartaric acid in saline (pH adjusted to 4.0 with 1 M NaOH). The exact dose for each drug was achieved regardless of weight gain of the animals by weighing the rats every week and adjusting the amount of drug in solution accordingly. Animals were decapitated 24 h following last drug administration (between 0900 and 1200), blood trunk samples were collected to determine the concentration of drug and their brains dissected on ice. PFC was collected and snap frozen on dry ice as described by Tilleman et al., 2002. The striatum and VTA were isolated and frozen at -85°C for subsequent synaptoneurosomal protein extraction.

4.2. Synaptoneurosomal preparations

Synaptoneurosomal-enriched fractions were prepared according to an adaptation to the protocol developed by Hollingsworth, Villasana and collaborators (Hollingsworth et al., 1985; Villasana et al., 2006). PFC from each treated rat and control was weighed, homogenized in 500 μ l synaptoneurosomal buffer (10 mM HEPES, 1 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 10 μ g/ml Leupeptin and 50 μ g/ml soybean trypsin inhibitor, pH 7.0) at 4°C using a Teflon-glass mechanical tissue grinder (0.25 mm clearance). The homogenate was diluted further with the same volume of synaptoneurosomal buffer and briefly and gently sonicated using a 60 sonic dismembrator (Fisher Scientific, Pittsburg, PA) delivering 3 pulses using an output power of 1 (dial setting 1). The resulting solution was loaded into a 3 ml syringe and filtered once through one layer of pre-wetted 180 μ m pore nylon filters (Millipore, Billerica, MA), held in a 13 mm diameter filter holder. The filtrate was loaded into a 5 ml syringe and filtered once through a 60 μ m pore nylon

filter (Millipore), divided into small fractions and filtered through a 5 μm filter (National Scientific Company, Rockwood, TN), pooled and centrifuged at 1000 000 $\times g$ for 10 min. An aliquot of the supernatant was saved for Western blot analysis. The resulting pellet containing the synaptoneurosomal-enriched preparation was re-dissolved in a small amount of synaptoneurosomal buffer and centrifuged again at 1000 000 $\times g$ for 10 min. The resulting synaptoneurosomal-enriched pellets were saved for further analysis. Aliquots of the initial protein homogenate and the filtrate from each step were saved to determine protein concentration and for Western blot analysis to confirm the presence of synaptoneurosomal proteins. Protein concentration was determined using the BCA assay (Pierce, Rockford, IL) and samples were stored until analyzed by two-dimensional fluorescence differential gel electrophoresis (2D-DIGE).

4.3. Western blots

To confirm enrichment with synaptoneurosomal proteins, each step of the isolation of synaptoneurosomal-enriched pellets was followed by Western blot. Proteins extracted above were loaded (50 mg/well) in 4–12% gradient Bis-Tris gels (Nupage-Invitrogen, Carlsbad, CA) and SDS-PAGE gel electrophoresis was performed following the manufacturers instructions for 150 V constant for 1 h. Proteins were transferred to a Nylon membrane (Millipore, Billerica, MA), using 30 V at 4 °C for 4 h. After transfer was completed, the blot was rinsed with Tris-buffered saline (TBS) pH 7.4, incubated for 30 min in blocking solution (20 g dry powdered milk, 400 ml 1 \times TBS, 400 μl goat serum, and 200 μl Tween 20) followed by three consecutive washes of 15 min each with TBS. After rinsing the blot was incubated in primary antibodies in blocking solution as described by Villasana and collaborators (Villasana et al., 2006). Antibodies included anti-glial fibrillary acidic protein of 50 kD (GFAP-50, a glial marker), anti-postsynaptic density protein of 95 kD (PSD-95, a postsynaptic protein marker), and anti-calcium/calmodulin-dependent protein kinase II (CaMKII-60, a pansynaptic marker). Anti-GFAP-50 (a gift from Dr. Paul J. Linser from the Whitney Laboratory for Marine Bioscience, St. Augustine, FL) was diluted 1:1500, anti-PSD-95 (Sigma) diluted 1:2000, and anti-CaMKII-60 (Chemicon International, Temecula, CA) diluted 1:2000. Horseradish peroxidase-conjugated secondary antibodies (goat anti-mouse) diluted at 1:2000 were obtained from Promega (Madison, WI, USA). Blots were washed in TBS three consecutive times for 5 min each and visualized using Enhanced chemiluminescence (ECL) Western blotting detection from Amersham Biosciences (Piscataway, NJ, USA) and Kodak Biomax film (Kodak, Rochester, NY).

4.4. 2D DIGE

2D-DIGE was performed by Applied Biomics (Hayward, CA) following established protocols. In detail, 2D lysis buffer (30 mM Tris-HCl, pH 8.8, 7 M Urea, 2 M thio-urea, and 4% CHAPS) was added to each synaptoneurosomal pellet and sonicated for 5 s using VirSonic 100 (VirTis) at power level 4. After vigorous shaking at RT for 30 min., the lysates were cleared at 16,000 $\times g$ for 30 min. Supernatant was transferred to

Eppendorf tubes. Protein concentration was adjusted to 5 mg/ml for each sample with lysis buffer.

30 μg of synaptoneurosomal preparation was labeled with 0.7 μl CyDye dilution (Cy2, Cy3, and Cy5, Amersham, Piscataway, NJ) per group. The CyDyes were diluted 1:5 with dimethylformamide (DMF) before each reaction, incubated on ice for 30 min, followed by 0.7 μl of 10 mM lysine to stop the labeling reaction. The final mix was kept on ice in the dark for 15 min. The CyDye-labeled preparations were mixed and an equal volume of 2 \times 2D sample buffer (8 M urea, 4% CHAPS, 20 mg/ml DTT, 2% pharmalytes, and trace amount of bromophenol blue) was added followed by 100 μl destreak solution (GE Healthcare, Piscataway, NJ) and rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 20 mg/ml DTT, 1% pharmalytes and trace amount of bromophenol blue) was added to a total volume of 260 μl . Solutions were incubated at RT for 10 min on a shaker and centrifuged for 10 min at 16,000 000 $\times g$ before loading 250 μl per immobilized pH gradient (IPG) strip [13 cm, pH 3–10 linear isoelectric focusing (IEF) strip, from Amersham]. IEF was performed for a total of 25000 V-h using standard conditions (Amersham). Each IPG strip was incubated with 10 ml of equilibration solution 1 (50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, trace amount of bromophenol blue, and 10 mg/ml DTT) for 15 min with gentle shaking followed by incubation in 10 ml of equilibration solution 2 (50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, trace amount of bromophenol blue, and 45 mg/ml iodoacetamide) for 10 min with gentle shaking. Strips were rinsed in SDS gel running buffer once and inserted into a 10.5% SDS gel prepared using low fluorescent glass plates (18 \times 16 cm, 1 mm thickness) and sealed with 0.5% agarose sealing solution in SDS running buffer. Electrophoresis was performed at 16 °C. Each gel was scanned immediately following electrophoresis using a Typhoon Trio Scanner (Amersham). Images were analyzed using ImageQuant software.

4.5. Image analysis and spot detection

Further analysis and quantification were done with DeCyder software (Ludesi, Lund, Sweden). Spot detection and matching were generated by the software and each spot was manually checked for accuracy. Differences in protein expression were determined using Ludesi's 2D Interpreter. Minimum protein volume was set at 200 and only those proteins with a 2 fold or more difference in protein expression, a 100% presence in all gel images, and P -values < 0.05 (ANOVA) were selected. Hierarchical clustering (HC) and Support Vector Machines (SVM) were used to classify selected proteins into groups. Groups of images were created per treatment group. Analysis of expression was performed independently by comparing each treatment separately and all sets of gels to the saline control group. The paliperidone and saline group were compared to the tartaric acid group to determine changes in expression caused by the addition of acid to aid in solubilization of the drug. Unsupervised and supervised HC were performed. For the supervised HC, 75% of the 20 gel images in each group were used as training sets and all gels were analyzed as test sets. Results of both supervised and unsupervised HC were compared and a list of proteins of

interest (POI) was generated. Pearson correlation and average linking methods were used to determine distance function and to cluster the classifying proteins. One degree polynomial analysis was used for SVM. Protein spots showing significant changes in mean normalized quantity from the POI were selected. Each spot was verified by manual comparison of the three sets of gels before being picked and identified by nano LC-MS/MS. Protein spots were excised from the gel using Ettan spot picker (Amersham) and sent to the Proteomics Research Center at the Mayo Clinic in Rochester, MN for further analysis as described next.

4.6. Trypsin digestion and nanoLC-ESI tandem mass spectrometry

Excised spots were reduced with 20 mM DTT/50 mM Tris-HCl, pH 8.1 at 55 °C for 30 min and alkylated with 40 mM iodoacetamide at RT for 30 min in the dark. Proteins were digested *in-situ* with 10 μ l (0.002 μ g/ μ l) trypsin (Promega Corporation, Madison WI) in 20 mM Tris-HCl, pH 8.1/0.0002% Zwittergent 3–16, at 37 °C overnight followed by peptide extraction with 20 μ l of 2% trifluoroacetic acid and 40 μ l of acetonitrile. Pooled extracts were concentrated to less than 5 μ l on a SpeedVac spinning concentrator (Savant Instruments, Holbrook NY) and then brought up in 0.1% formic acid for protein identification by nano-flow liquid chromatography, electrospray tandem mass spectrometry (nano LC-ESI-MS/MS) with the use of a ThermoFinnigan LTQ Orbitrap Hybrid Mass Spectrometer (ThermoElectron Bremen, Germany) coupled to an Eksigent nanoLC-2D HPLC system (Eksigent, Dublin, CA). The peptide mixture was loaded onto a 250 nl OPTI-PAK trap (Optimize Technologies, Oregon City, OR) custom packed with Michrom Magic C8 solid phase (Michrom Bioresources, Auburn, CA) and eluted with a 0.2% formic acid/acetonitrile gradient through a Michrom packed tip capillary Magic C18 column (75 μ m \times 150 mm).

The LTQ Orbitrap mass spectrometer performed a Fourier Transformed (FT) full scan from 380–1600 m/z with resolving power set at 60,000 (400 m/z), followed by linear ion trap MS/MS scans on the top 3 ions. Dynamic exclusion was set to 2 and selected ions were placed on an exclusion list for 60 s. The MS/MS raw data were converted to DTA files using ThermoElectron Bioworks 3.2 and correlated to theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot databases using both SEQUEST™ (ThermoElectron, San Jose, CA) and MASCOT™ (Matrix Sciences London, UK) search algorithms running on 10 node clusters. All searches were conducted with fixed cysteine modifications of +57 for carboxamidomethyl-cysteines and variable modifications allowing +16 with methionines for methionine sulphoxide, and +42 for protein N-terminal acetylation. The search was restricted to trypsin generated peptides allowing for 2 missed cleavages and is open to all species. Peptide mass search tolerances were set to 10 ppm and fragment mass tolerance was set to \pm 0.8 Da. Protein identifications were considered when both Mascot and Sequest gave at least two consensus peptides with individual cross correlation scores exceeding 2.2 for +2 peptides or 3.2 for +3 peptides and >95% probability scores in addition to ranking as the number one hit for their respective MS/MS spectra.

Acknowledgments

We would like to thank Dr. Eric de Waal for his advice on paliperidone solubility and Albin Olofsson M.Sc. for his valuable help with Ludesi software. Our special gratitude to Mr. James Tidwell for his valuable help with illustrations.

REFERENCES

- Ackerley, S., Thornhill, P., Grierson, A.J., Brownlees, J., Anderton, B.H., Leigh, P.N., Shaw, C.E., Miller, C.C., 2003. Neurofilament heavy chain side arm phosphorylation regulates axonal transport of neurofilaments. *J. Cell Biol.* 161, 489–495.
- Alimohamad, H., Rajakumar, N., Seah, Y.H., Rushlow, W., 2005. Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. *Biol. Psychiatry.* 57, 533–542.
- Arthur, J.R., Nicol, F., Beckett, G.J., 1993. Selenium deficiency, thyroid hormone metabolism, and thyroid hormone deiodinases. *Am. J. Clin. Nutr.* 57, 236S–239S.
- Bai, J., Cederbaum, A.I., 2000. Overexpression of catalase in the mitochondrial or cytosolic compartment increases sensitivity of HepG2 cells to tumor necrosis factor- α -induced apoptosis. *J. Biol. Chem.* 275, 19241–19249.
- Baxter Jr., L.R., Schwartz, J.M., Phelps, M.E., Mazziotta, J.C., Guze, B.H., Selin, C.E., Gerner, R.H., Sumida, R.M., 1989. Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Arch. Gen. Psychiatry.* 46, 243–250.
- Bennett, A.F., Hayes, N.V., Baines, A.J., 1991. Site specificity in the interactions of synapsin 1 with tubulin. *Biochem. J.* 276 (Pt 3), 793–799.
- Blumberg, H.P., Kaufman, J., Martin, A., Whiteman, R., Zhang, J.H., Gore, J.C., Charney, D.S., Krystal, J.H., Peterson, B.S., 2003. Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder. *Arch. Gen. Psychiatry.* 60, 1201–1208.
- Blumberg, H.P., Stern, E., Ricketts, S., Martinez, D., de Asis, J., White, T., Epstein, J., Isenberg, N., McBride, P.A., Kemperman, I., Emmerich, S., Dhawan, V., Eidelberg, D., Kocsis, J.H., Silbersweig, D.A., 1999. Rostral and orbital prefrontal cortex dysfunction in the manic state of bipolar disorder. *Am. J. Psychiatry.* 156, 1986–1988.
- Bollen, M., Stalmans, W., 1992. The structure, role, and regulation of type 1 protein phosphatases. *Crit. Rev. Biochem. Mol. Biol.* 27, 227–281.
- Bradshaw, J.M., Kubota, Y., Meyer, T., Schulman, H., 2003. An ultrasensitive Ca²⁺/calmodulin-dependent protein kinase II-protein phosphatase 1 switch facilitates specificity in postsynaptic calcium signaling. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10512–10517.
- Butterfield, D.A., Perluigi, M., Sultana, R., 2006. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. *Eur. J. Pharmacol.* 545, 39–50.
- Carboni, L., Vighini, M., Piubelli, C., Castelletti, L., Milli, A., Domenici, E., 2006. Proteomic analysis of rat hippocampus and frontal cortex after chronic treatment with fluoxetine or putative novel antidepressants: CRF1 and NK1 receptor antagonists. *Eur. Neuropsychopharmacol.* 16, 521–537.
- Chen, G., Huang, L.D., Jiang, Y.M., Manji, H.K., 1999. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *J. Neurochem.* 72, 1327–1330.
- Dou, H., Biringh, K., Faraci, J., Gorantla, S., Poluektova, L.Y., Maggirwar, S.B., Dewhurst, S., Gelbard, H.A., Gendelman, H.E., 2003. Neuroprotective activities of sodium valproate in a

- murine model of human immunodeficiency virus-1 encephalitis. *J. Neurosci.* 23, 9162–9170.
- Dremencov, E., El Mansari, M., Blier, P., 2007. Distinct electrophysiological effects of paliperidone and risperidone on the firing activity of rat serotonin and norepinephrine neurons. *Psychopharmacology (Berl)* 194, 63–72.
- Drigues, N., Poltyrev, T., Bejar, C., Weinstock, M., Youdim, M.B., 2003. cDNA gene expression profile of rat hippocampus after chronic treatment with antidepressant drugs. *J. Neural. Transm.* 110, 1413–1436.
- Ferkey, D.M., Kimelman, D., 2000. GSK-3: new thoughts on an old enzyme. *Dev. Biol.* 225, 471–479.
- Frame, S., Cohen, P., 2001. GSK3 takes centre stage more than 20 years after its discovery. *Biochem J* 359, 1–16.
- Goold, R.G., Owen, R., Gordon-Weeks, P.R., 1999. Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. *J. Cell Sci.* 112 (Pt 19), 3373–3384.
- Greenough, W.T., Klintsova, A.Y., Irwin, S.A., Galvez, R., Bates, K.E., Weiler, I.J., 2001. Synaptic regulation of protein synthesis and the fragile X protein. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7101–7106.
- Haldane, M., Frangou, S., 2004. New insights help define the pathophysiology of bipolar affective disorder: neuroimaging and neuropathology findings. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 28, 943–960.
- Hall, A.C., Brennan, A., Goold, R.G., Cleverley, K., Lucas, F.R., Gordon-Weeks, P.R., Salinas, P.C., 2002. Valproate regulates GSK-3-mediated axonal remodeling and synapsin I clustering in developing neurons. *Mol. Cell. Neurosci.* 20, 257–270.
- Hall, A.C., Lucas, F.R., Salinas, P.C., 2000. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* 100, 525–535.
- Hayden, E.P., Nurnberger Jr., J.I., 2006. Molecular genetics of bipolar disorder. *Genes. Brain Behav.* 5, 85–95.
- Hodel, A., 1998. Snap-25. *Int. J. Biochem. Cell Biol.* 30, 1069–1073.
- Hollingsworth, E.B., McNeal, E.T., Burton, J.L., Williams, R.J., Daly, J.W., Creveling, C.R., 1985. Biochemical characterization of a filtered synaptoneurosome preparation from guinea pig cerebral cortex: cyclic adenosine 3':5'-monophosphate-generating systems, receptors, and enzymes. *J. Neurosci.* 5, 2240–2253.
- Illig, A.M., Melia, K., Snyder, P.J., Badura, L.L., 2000. Sodium valproate alters GnRH-GABA interactions during development in seizure-prone mice. *Brain Res.* 885, 192–200.
- Jovanovic, J.N., Czernik, A.J., Fienberg, A.A., Greengard, P., Sihra, T.S., 2000. Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nat. Neurosci.* 3, 323–329.
- Kamma, H., Horiguchi, H., Wan, L., Matsui, M., Fujiwara, M., Fujimoto, M., Yazawa, T., Dreyfuss, G., 1999. Molecular characterization of the hnRNP A2/B1 proteins: tissue-specific expression and novel isoforms. *Exp. Cell Res.* 246, 399–411.
- Karry, R., Klein, E., Ben Shachar, D., 2004. Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. *Biol. Psychiatry.* 55, 676–684.
- Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8455–8459.
- Konradi, C., Eaton, M., MacDonald, M.L., Walsh, J., Benes, F.M., Heckers, S., 2004. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch. Gen. Psychiatry.* 61, 300–308.
- Kronhaus, D.M., Lawrence, N.S., Williams, A.M., Frangou, S., Brammer, M.J., Williams, S.C., Andrew, C.M., Phillips, M.L., 2006. Stroop performance in bipolar disorder: further evidence for abnormalities in the ventral prefrontal cortex. *Bipolar. Disord.* 8, 28–39.
- Lemasters, J.J., Holmuhamedov, E., 2006. Voltage-dependent anion channel (VDAC) as mitochondrial governor-thinking outside the box. *Biochim. Biophys. Acta* 1762, 181–190.
- Li, K.W., Hornshaw, M.P., Van Der Schors, R.C., Watson, R., Tate, S., Casetta, B., Jimenez, C.R., Gouwenberg, Y., Gundelfinger, E.D., Smalla, K.H., Smit, A.B., 2004. Proteomics analysis of rat brain postsynaptic density. Implications of the diverse protein functional groups for the integration of synaptic physiology. *J. Biol. Chem.* 279, 987–1002.
- Li, X., Rosborough, K.M., Friedman, A.B., Zhu, W., Roth, K.A., 2007. Regulation of mouse brain glycogen synthase kinase-3 by atypical antipsychotics. *Int J Neuropsychopharmacol* 10, 7–19.
- Longtine, M.S., DeMarini, D.J., Valencik, M.L., Al-Awar, O.S., Fares, H., De Virgilio, C., Pringle, J.R., 1996. The septins: roles in cytokinesis and other processes. *Curr. Opin. Cell Biol.* 8, 106–119.
- Lucas, F.R., Goold, R.G., Gordon-Weeks, P.R., Salinas, P.C., 1998. Inhibition of GSK-3beta leading to the loss of phosphorylated MAP-1B is an early event in axonal remodelling induced by WNT-7a or lithium. *J. Cell Sci.* 111 (Pt 10), 1351–1361.
- Lucas, F.R., Salinas, P.C., 1997. WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. *Dev. Biol.* 192, 31–44.
- Manji, H.K., Moore, G.J., Chen, G., 2000. Lithium up-regulates the cytoprotective protein Bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. *J. Clin. Psychiatry.* 61 Suppl. 9, 82–96.
- Morfino, G., Szebenyi, G., Brown, H., Pant, H.C., Pigino, G., DeBoer, S., Beffert, U., Brady, S.T., 2004. A novel CDK5-dependent pathway for regulating GSK3 activity and inosin-driven motility in neurons. *Embo. J.* 23, 2235–2245.
- Nagy, G., Matti, U., Nehring, R.B., Binz, T., Rettig, J., Neher, E., Sorensen, J.B., 2002. Protein kinase C-dependent phosphorylation of synaptosome-associated protein of 25 kDa at Ser187 potentiates vesicle recruitment. *J. Neurosci.* 22, 9278–9286.
- O'Leary, G., Bacon, C.L., Odumeru, O., Fagan, C., Fitzpatrick, T., Gallagher, H.C., Moriarty, D.C., Regan, C.M., 2000. Antiproliferative actions of inhalational anesthetics: comparisons to the valproate teratogen. *Int. J. Dev. Neurosci.* 18, 39–45.
- Ogden, C.A., Rich, M.E., Schork, N.J., Paulus, M.P., Geyer, M.A., Lohr, J.B., Kuczenski, R., Niculescu, A.B., 2004. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol. Psychiatry.* 9, 1007–1029.
- Perez, M., Rojo, A.I., Wandosell, F., Diaz-Nido, J., Avila, J., 2003. Prion peptide induces neuronal cell death through a pathway involving glycogen synthase kinase 3. *Biochem. J.* 372, 129–136.
- Quraishi, S., Frangou, S., 2002. Neuropsychology of bipolar disorder: a review. *J. Affect. Disord.* 72, 209–226.
- Raabe, T.D., Nguyen, T., Archer, C., Bittner, G.D., 1996. Mechanisms for the maintenance and eventual degradation of neurofilament proteins in the distal segments of severed goldfish mauthner axons. *J. Neurosci.* 16, 1605–1613.
- Renouf, S., Beullens, M., Wera, S., Van Eynde, A., Sikela, J., Stalmans, W., Bollen, M., 1995. Molecular cloning of a human polypeptide related to yeast sds22, a regulator of protein phosphatase-1. *FEBS Lett.* 375, 75–78.
- Richelson, E., Souder, T., 2000. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. *Life Sci* 68, 29–39.
- Rubinsztein, J.S., Sahakian, B.J., 2002. Cognitive impairment in bipolar disorder. *Br. J. Psychiatry.* 181, 440.
- Schrimpf, S.P., Meskenaite, V., Brunner, E., Rutishauser, D., Walther, P., Eng, J., Aebersold, R., Sonderegger, P., 2005. Proteomic analysis of synaptosomes using isotope-coded affinity tags and mass spectrometry. *Proteomics* 5, 2531–2541.
- Shields, S.M., Ingebritsen, T.S., Kelly, P.T., 1985. Identification of protein phosphatase 1 in synaptic junctions:

- dephosphorylation of endogenous calmodulin-dependent kinase II and synapse-enriched phosphoproteins. *J. Neurosci.* 5, 3414–3422.
- Shoji-Kasai, Y., Itakura, M., Kataoka, M., Yamamori, S., Takahashi, M., 2002. Protein kinase C-mediated translocation of secretory vesicles to plasma membrane and enhancement of neurotransmitter release from PC12 cells. *Eur. J. Neurosci.* 15, 1390–1394.
- Stone, E.M., Yamano, H., Kinoshita, N., Yanagida, M., 1993. Mitotic regulation of protein phosphatases by the fission yeast sds22 protein. *Curr. Biol.* 3, 13–26.
- Tariot, P.N., Loy, R., Ryan, J.M., Porsteinsson, A., Ismail, S., 2002. Mood stabilizers in Alzheimer's disease: symptomatic and neuroprotective rationales. *Adv. Drug Deliv. Rev.* 54, 1567–1577.
- Thomas, J.P., Maiorino, M., Ursini, F., Girotti, A.W., 1990. Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation. In situ reduction of phospholipid and cholesterol hydroperoxides. *J. Biol. Chem.* 265, 454–461.
- Tilleman, K., Van den Haute, C., Geerts, H., van Leuven, F., Esmans, E.L., Moens, L., 2002. Proteomics analysis of the neurodegeneration in the brain of tau transgenic mice. *Proteomics* 2, 656–665.
- Tohda, M., Qi, Z., Watanabe, H., 1999. Influence of chronic treatment with imipramine on mRNA levels in rat brain: elevation of glyceraldehyde-3-phosphate dehydrogenase levels. *Jpn. J. Pharmacol.* 81, 393–396.
- Villasana, L.E., Klann, E., Tejada-Simon, M.V., 2006. Rapid isolation of synaptoneuroosomes and postsynaptic densities from adult mouse hippocampus. *J. Neurosci. Methods* 158, 30–36.
- Witzmann, F.A., Arnold, R.J., Bai, F., Hrnčirova, P., Kimpel, M.W., Mechref, Y.S., McBride, W.J., Novotny, M.V., Pedrick, N.M., Ringham, H.N., Simon, J.R., 2005. A proteomic survey of rat cerebral cortical synaptosomes. *Proteomics* 5, 2177–2201.
- Wu, K., Aoki, C., Elste, A., Rogalski-Wilk, A.A., Siekevitz, P., 1997. The synthesis of ATP by glycolytic enzymes in the postsynaptic density and the effect of endogenously generated nitric oxide. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13273–13278.
- Yagi, K., Komura, S., Kojima, H., Sun, Q., Nagata, N., Ohishi, N., Nishikimi, M., 1996. Expression of human phospholipid hydroperoxide glutathione peroxidase gene for protection of host cells from lipid hydroperoxide-mediated injury. *Biochem. Biophys. Res. Commun.* 219, 486–491.
- Zhang, F., Phiel, C.J., Spece, L., Gurvich, N., Klein, P.S., 2003. Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3. *J. Biol. Chem.* 278, 33067–33077.