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SHORT COMMUNICATION

Proteome analyses of cultured astrocytes treated with MK-801 and clozapine: similarities with schizophrenia

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Abstract On the basis of impaired glutamatergic transmission and the potential role of astrocytes in schizophrenia, we treated cultured astrocytes with MK-801, an NMDA-receptor antagonist, to investigate whether the resulting proteome changes are similar to those we found in our earlier proteome analysis of schizophrenia human brain tissue as well as to better comprehend the role of astrocytes in the disorder. Indeed, there are similarities. Furthermore, to verify the efficacy of clozapine and its effect over the proteome, we treated MK-801-treated astrocytes with clozapine. Interestingly, clozapine reversed protein changes induced by MK-801. The treatment of cell cultures with neural transmission agonists and antagonists might provide useful insights about psychiatric disorders.

Keywords Proteome · Proteomics · Astrocytes · Biomarkers · Mass spectrometry · Schizophrenia

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Introduction

Although the molecular background of schizophrenia (SCZ) is not completely understood, some evidence has been found for the impairment of glutamatergic transmission [23, 27] and a role of glial cells such as astrocytes [4].

MK-801 (or dizocilpine) is a non-competitive NMDA-receptor antagonist that binds to the NMDA receptor, preventing the flow of ions such as calcium (Ca^{2+}) [6]. NMDA-receptor antagonists, such as MK-801 and phencyclidine, are hypothesized not only to aggravate SCZ symptoms but also to induce symptoms similar to SCZ positive and negative symptoms [29]. In this case, in vivo treatments with NMDA-receptor antagonists would be better models of SCZ than treatments with dopaminergic agonists, which might mimic positive symptoms only [25]. MK-801-treated rats have been used as a candidate model of SCZ [8, 22], and their proteome has been studied [21]. As an emerging and effective technique for the systemic investigation of complex pathogenic processes such as SCZ, proteomics may identify quantitative and qualitative protein expression differences between two or more states.

Astrocytes play a role not only in the structural stability of brain tissue but also in water and electrolyte homeostasis, provision of nutrients to neurons and other glial cells, neuronal migration, repair processes, modulation of the immune response and neurotransmitter release, and control of synapse formation [9]. Despite some controversial results, astrocytes have emerged as important players in the pathobiology of SCZ: the differential expression of genes and proteins was found to be astrocytes-specific, and astrocytes are known to play a key role in the synaptic metabolism of glutamate and monamines [4].

Here, we present the results of a proteome analysis that used two-dimensional gel electrophoresis (2-DE) for

protein separation and MALDI-TOF/TOF mass spectrometry (MS) for protein identification comparing cultured MK-801-treated astrocytes with non-MK-801-treated astrocytes (controls) at different concentrations and exposure times to investigate whether resulting proteome differences are similar to those in our earlier proteome analysis of SCZ human brain tissue [1, 2, 7, 13–19, 24, 28]. Moreover, we treated the cultured astrocytes with MK-801 and clozapine to verify clozapine's efficacy in reversing changes induced by MK-801. As a verification step, we treated astrocytes only with clozapine. The advantage of this study is to access exactly specific proteome differences of the astrocytes, what is not possible using brains from rats models. Our main aim is to understand in a proteomic context the potential role of astrocytes in SCZ.

Materials and methods

Cell culture and treatments with MK-801 and clozapine

Astrocytes (cell line 1321N1) were seeded in 250-ml flasks at a density of 1.8×10^5 cells/ml and grown as a monolayer (37°C, 5% CO_2 atmosphere) in 20-ml Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 containing penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM), supplemented with 5% (v/v) FBS (PAA laboratories, Pasching, Austria). The cultured astrocytes were treated with MK-801 and clozapine (both from Sigma–Aldrich, St Louis, MO, USA) under two different conditions: acute treatment, in which the cultured astrocyte samples were collected 8 h after treatment; and chronic treatment, in which they were collected 24 h and 72 h after treatment. In the chronic treatment, cells were treated every 24 h with the same concentration of MK-801 or clozapine or both. The controls were treated with the vehicle (for MK-801: water; for clozapine: 0.01 M HCl) in which, according to the manufacturer, the compounds were dissolved. In the acute treatment, cultured astrocytes were treated with 20 or 50 μM MK-801 or 50 μM clozapine. In the chronic treatment, astrocytes were treated with 10, 17.5, or 25 μM MK-801 or 10 μM clozapine. Concentrations were based on those of previous studies [11, 20, 21]. MK-801 (10 μM) and clozapine (10 μM) were administered together only in a chronic treatment: in one experiment, both were administered at the same time, once a day; in the other, clozapine was added 8 h after MK-801 and this procedure was repeated once a day. Those experiments were performed in duplicates.

Proteome analyses

Proteins from treated astrocytes were extracted as previously described [12]. Protein samples were separated by 2-DE as previously described [13] but using 11-cm IPG gel strips. Gels were scanned and the images analyzed using the software PDQuest (BioRad). Spot volumes were analyzed by Kruskal–Wallis one-way analysis of variance aiming to reveal the statistically significant differences in the protein expression between the groups. Protein spots with a mean *n*-fold change of ±1.8 were excised for MS identification as previously described in detail [13]. Experiments were performed in duplicates. Differentially expressed proteins were classified according to their main biological processes as well as their molecular functions using Human Protein Reference Database (HPRD—http://www.hprd.org).

Results

Compared with the control culture, astrocytes acutely treated with 20 μM MK-801 showed 11 differentially expressed proteins and those acutely treated with 50 μM showed 30 (Table 1). The differentially expressed proteins play roles most often in cell growth and/or maintenance (28%) or energy pathways (24%) (Suppl. Material 1A). Moreover, 10 of these proteins (33.3%) also were found differentially expressed in distinct regions of SCZ human brain tissue [19] (Table 1). Some of the differences in protein expression are represented in Fig. 1a.

In the chronic treatment with 25 μM MK-801, we observed a constant decrease in the cell population after 36 h and therefore abandoned this treatment because it was visibly harmful to the cells. The expression of many chaperones led us also to abandon the 17.5 μM chronic treatment because of the compromised homeostasis in the culture. In the 10 μM MK-801 chronic treatment, we found 18 differentially expressed proteins (Table 2), which play roles most often in energy metabolism pathways (43%) (Suppl. Material 1B). Moreover, 8 of these proteins (44.4%) also were found differentially expressed in SCZ human brain tissue [19] and 11 (61.1%) in the MK-801 acute treatment. We validated by Western blot the differential expression of GAPDH in MK-801 acute- and chronic-treated cells (Fig. 1b), since this is a protein previously found differentially expressed in SCZ brain tissue.

Cultured astrocytes were acutely treated (8 h) with 50 μM clozapine and chronically treated (72 h) with 10 μM. In total, eight proteins were identified as differentially expressed compared with the respective control cultures; 2 of these 8 had been found differentially expressed in SCZ human brain tissue [19] (Table 3). The differentially expressed proteins play roles most often in cell growth and/or maintenance (37.5%) or energy pathways (25%) (Suppl. Material 1C).

Finally, cultured astrocytes were chronically treated (72 h) with 10 μM of MK-801 and 10 μM clozapine was

Table 1 Differentially expressed proteins after acute treatment with 50 μM MK-801

Gene name	Protein name	Biological process	Molecular function	Uniprot access	MW	pI	Regulation in control	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
GANAB	Neutral alpha-glucosidase	Carbohydrate metabolism	Hydrolase activity	GANAB_HUMAN	107263	5.74	↓	118	24	30	
ANXA5	Annexin A5	Cell communication; signal transduction	Calcium ion binding	ANXA5_HUMAN	35971	4.94	↑	129	21	65	
YWHAQ	14-3-3 protein theta	Cell communication; signal transduction	Receptor signaling complex scaffold activity	1433T_HUMAN	28032	4.68	↑	94	17	47	
CALU	Calumenin	Cell communication; signal transduction	Calcium ion binding	CALU_HUMAN	37198	4.47	↑	94	11	37	
ANXA1	**Annexin A1**	**Cell communication; signal transduction**	**Calcium ion binding**	**ANXA1_HUMAN**	**38918**	**6.57**	↓	**226**	**14**	**50**	
SYT17	**Synaptotagmin-17**	**Cell communication; Signal transduction**	**Calcium ion binding**	**SYT17_HUMAN**	**54443**	**7.23**	↓	**67**	**14**	**28**	
TUBA1A	Tubulin alpha-1A chain	Cell growth and/or maintenance	Structural constituent of cytoskeleton	TBA1A_HUMAN	50788	4.94	↓	93	23	61	
TUBA1B	Tubulin alpha-1B chain	Cell growth and/or maintenance	Structural constituent of cytoskeleton	TBA1B_HUMAN	50804	4.94	↓	93	23	61	[24] (↓) [1] (↓) [28] (↓) [7] (↓) [19] (↓ and ↑)
TUBA1C	Tubulin alpha-1C chain	Cell growth and/or maintenance	Structural constituent of cytoskeleton	TBA1C_HUMAN	50548	4.96	↓	84	21	56	
TUBA4A	Tubulin alpha-4A chain	Cell growth and/or maintenance	Structural constituent of cytoskeleton	TBA4A_HUMAN	50634	4.95	↓	83	21	55	
VIM	Vimentin	Cell growth and/or maintenance	Structural constituent of cytoskeleton	VIME_HUMAN	53676	5.06	↓	228	35	69	[14] (↑) [18] (↑)
CAPZB	F-actin-capping protein subunit beta	Cell growth and/or maintenance	Structural constituent of cytoskeleton	CAPZB_HUMAN	31616	5.36	↑	101	14	47	
ACTG1	**Actin, cytoplasmic 2**	**Cell growth and/or maintenance**	**Structural constituent of cytoskeleton**	**ACTG_HUMAN**	**42108**	**5.31**	↓	**117**	**26**	**69**	**[13] (↓)** **[17] (↑)**
ACTB	**Actin, cytoplasmic 1**	**Cell growth and/or maintenance**	**Structural constituent of cytoskeleton**	**ACTB_HUMAN**	**42052**	**5.29**	↓	**106**	**24**	**64**	**[17] (↑)**

Table 1 continued

Gene name	Protein name	Biological process	Molecular function	Uniprot access	MW	pI	Regulation in control	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
STMN1	Stathmin	Cell growth and/or maintenance; signal transduction	Signal transducer activity	STMN1_HUMAN	17292	5.76	↑	58	7	48	
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism; energy pathways	Catalytic activity	G3P_HUMAN	36201	8.57	↓	81	7	28	[24] (↓) [16] (↓) [18] (↑)
PCK2	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	Metabolism; energy pathways	Catalytic activity	PCKGM_HUMAN	71447	7.56	↑	124	21	37	
PGK1	Phosphoglycerate kinase 1	Metabolism; energy pathways	Catalytic activity	PGK1_HUMAN	44985	8.3	↓	136	20	50	[13] (↑)
ALDOA	Fructose-bisphosphate aldolase A	Metabolism; energy pathways	Lyase activity	ALDOA_HUMAN	39851	8.3	↓	279	32	82	[24] (↓)
PGD	**6-phosphogluconate dehydrogenase**	**Metabolism; energy pathways**	**Catalytic activity**	**6PGD_HUMAN**	**53619**	**6.8**	↑	**93**	**11**	**31**	
ENO2	**Gamma-enolase**	**Metabolism; energy pathways**	**Catalytic activity**	**ENOG_HUMAN**	**47269**	**4.91**	↑	**88**	**12**	**34**	[24] (↓) [28] (↓) [16] (↑)
LDHB	**L-lactate dehydrogenase B chain**	**Metabolism; energy pathways**	**Catalytic activity**	**LDHB_HUMAN**	**36900**	**5.71**	↓	**83**	**11**	**32**	
TXNDC12	Thioredoxin domain-containing protein 12	Protein metabolism	Catalytic activity	TXD12_HUMAN	19365	5.24	↑	63	6	39	
HSPB1	Heat shock protein beta-1	Protein metabolism	Chaperone activity	HSPB1_HUMAN	22826	5.98	↓	82	10	60	
UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Protein metabolism	Ubiquitin-specific protease activity	UCHL1_HUMAN	25151	5.33	↑	81	14	60	
CCT8	**T-complex protein 1 subunit theta**	**Protein metabolism**	**Chaperone activity**	**TCPQ_HUMAN**	**60153**	**5.42**	↑	**126**	**23**	**40**	
PDIA3	**Protein disulfide-isomerase A3**	**Protein metabolism**	**Isomerase activity**	**PDIA3_HUMAN**	**57146**	**5.98**	↓	**99**	**18**	**39**	
HSPA8	**Heat shock cognate 71 kDa protein**	**Protein metabolism**	**Heat shock protein activity**	**HSP7C_HUMAN**	**71082**	**5.37**	↓	**130**	**30**	**55**	[18] (↓)

Table 1 continued

Gene name	Protein name	Biological process	Molecular function	Uniprot access	MW	pI	Regulation in control	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
HNRPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA binding	ROA2_HUMAN	37430	8.46	↑	134	19	39	[18] (↓)
EEF1A1	**Elongation factor 1-alpha 1**	**Regulation of cell cycle**	**Transcription regulator activity**	**EF1A1_HUMAN**	**50451**	**9.1**	↓	**132**	**22**	**45**	

Bold cells indicate proteins that were found differentially expressed also after 20 μM treatment

added, either at the same time or 8 h later. When compared with the control culture, 5 differentially expressed proteins were found after simultaneous treatment and 3 after delayed treatment (Tables 4, 5), suggesting that clozapine reversed the major proteome differences induced by MK-801.

Discussion

The administration of MK-801 in rats has been suggested as a candidate model for SCZ [8, 22]. Although the main MK-801 role in the CNS is the antagonistic effect over NMDA receptors, this compound may cause other effects which need observation, such as a proteomic overview, in order to point out potential correlations with SCZ. Thus, the use of MK-801 in astrocyte cell cultures might allow not only the understanding of cellular and molecular mechanisms triggered by MK-801 that could be relevant in SCZ but also speculations to be made about the role of astrocytes in this disorder, considering astrocytic roles in the metabolism of glutamate and monamines [4].

Interestingly, 33.3% of the differentially expressed proteins in the acutely MK-801-treated astrocytes and 44.4% of those in the chronically treated ones also were found differentially expressed in SCZ human brain tissue (not only astrocytes), supporting MK-801 treatment as a potential model as previously described. Roughly, 22% of these proteins, e.g. GAPDH, PGK1, ALDOA, ENO2, PKM2, TKT, PRDX6, and ATP5A1, play roles in energy metabolism pathways and have been detected in different concentrations in dorsolateral prefrontal cortex, anterior temporal lobe, Wernicke's area, and anterior cingulate cortex from SCZ patients [1, 13–18, 24, 28]. Different lines of evidence have found that impairments in energy metabolism pathways may disturb other related pathways, such as calcium homeostasis and oxidative phosphorylation, and abnormal production of reactive oxygen species and apoptotic factors, which may ultimately affect neuronal processes such as neurotransmitter synthesis and synaptic plasticity.

A considerable number of the differentially expressed proteins in both MK-801-treated astrocytes (Tables 1, 2, 3) and SCZ human brain tissue [19] are involved in cytoskeleton arrangement, another aspect found disturbed in SCZ brain tissue [2, 7, 13].

The impairment of phospholipids metabolism also might be indicated by MK-801-treated astrocytes, since 3 isoforms of annexin (Annexin A1, A2, and A5) were found differentially expressed, as was PRDX6. Annexins inhibit phospholipase A2 (PLA2) activity [26], and PRDX6, through its PLA2 activity, helps regulate phospholipid turnover [5].

When we compared our findings with the proteome analyses of MK-801-treated rats, we found similarities such as differential expression of the energy metabolism

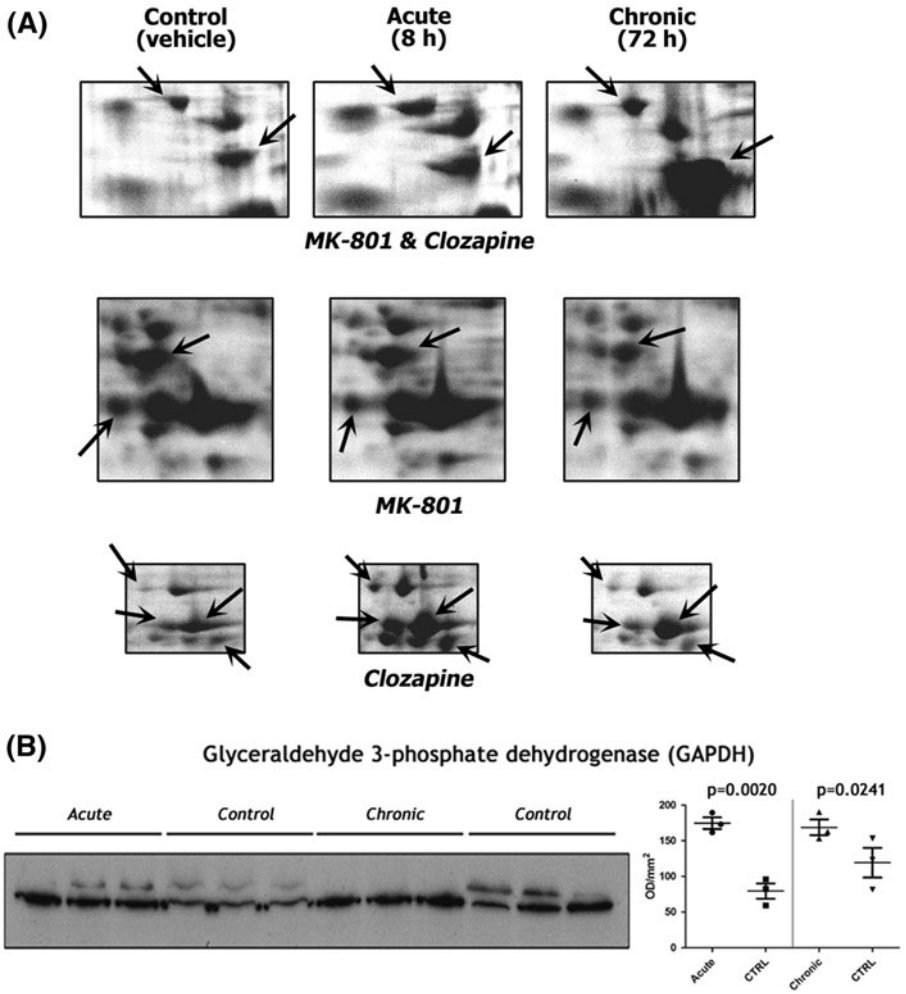

Fig. 1 **a** Enlarged sections of the 2-DE profiles representing the reproducibility of the gels as well as the differential protein expression from treated and control astrocytes. **b** Validation of the differentially expressed protein GAPDH found in MK-801 acute (8 h) and chronic (72 h) treatment using Western blot

proteins ENO2, ALDOA, and ATP-synthase and of structural proteins such as STMN1 and ACTB [21, 22], reinforcing the usefulness of MK-801 as a model for comparing and comprehend the mechanisms of SCZ.

Attempting to show the influence of antipsychotic medication on the proteome of cultured astrocytes, the astrocytes were acutely (50 μM) and chronically (10 μM) treated with clozapine. The differential expression of 8 proteins, most of them related to cell growth and/or maintenance, provided some information about the effect of antipsychotic treatment on astrocytes. Actin subunits beta and gamma previously were found differentially expressed in SCZ human brain tissue [13, 17], indicating the probable effects of medication on the proteome of SCZ patients who were indeed chronically treated with antipsychotics. However, these actins also were found differentially expressed in MK-801-treated rats and MK-801-cultured astrocytes, confirming the expected sensitive regulation of the cytoskeletal microfilaments.

When clozapine was added to MK-801-treated astrocytes, either simultaneously (Table 4) or 8 h later (Table 5), differential protein expression found in the MK-801 conditions without clozapine was practically reversed. Cultured astrocytes simultaneously treated with MK-801 and clozapine showed reduced proteome differences: alterations were found in only 2 actin subunits (also found in the separate MK-801 and clozapine treatments), 2 chaperones and 1 metabolic enzyme. Although both treatment conditions (simultaneous and delayed administration) appear to be effective in reversing proteome differences, delayed treatment with clozapine appears to be more beneficial—we found differential expression only of 1 chaperone and the same 2 actin isoforms, proteins that are expected to be altered in any disturbing situation, even a mild one which is the case here. The behavioral changes that MK-801 causes in rodents, probably resulting from an interaction between the glutamatergic and dopaminergic systems, were shown to be treatable with antipsychotics [3, 10, 30], supporting the proteome studies in MK-801 rats [20] and the results presented here. Moreover, the reversible effects of clozapine in the proteome level here described might suggest that the action of this drug goes beyond their actions on serotonin and dopamine receptors.

Table 2 Differentially expressed proteins after chronic treatment with 10 μM MK-801

Gene name	Protein name	Biological process	Molecular function	Uniprot access	MW	pI	Regulation control	Regulation 1 day	Regulation 3 days	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
YWHAQ*	14-3-3 protein theta	Cell communication; signal transduction	Receptor signaling complex scaffold activity	1433T_HUMAN	28032	4.68	↑	↓	↓	94	17	47	
ACTB*,#	Actin, cytoplasmic 1	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTB_HUMAN	42052	5.29	↓	↑	↑	106	24	64	[17] (↑)
ACTG1*,#	Actin, cytoplasmic 2	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTG_HUMAN	42108	5.31	↓	↑	↑	117	26	69	[13] (↓) [17] (↑)
FSCN1	Fascin 1	Cell growth and/or maintenance	Structural molecule activity	FSCN1_HUMAN	55123	6.84	↓	↑	↑	96	14	34	
ATP5A1	ATP-synthase subunit alpha, mitochondrial	Metabolism; energy pathways	Transporter activity	ATPA_HUMAN	59828	9.16	↑	↓	↓	84	17	43	[17] (↑) [16] (↓)
ENO2*	Gamma-enolase	Metabolism; energy pathways	Catalytic activity	ENOG_HUMAN	47269	4.91	↓	↑	↓	88	12	34	[24] (↓) [28] (↓) [16] (↑)
GAPDH*,#	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism; energy pathways	Catalytic activity	G3P_HUMAN	36201	8.57	↓	↓	↑	232	21	59	[24] (↓) [16] (↓) [18] (↑)
LDHB*	L-lactate dehydrogenase B chain	Metabolism; Energy pathways	Catalytic activity	LDHB_HUMAN	36900	5.71	↑	↓	↓	83	11	32	
PGD*	6-phosphogluconate dehydrogenase	Metabolism; Energy pathways	Catalytic activity	6PGD_HUMAN	53619	6.8	↓	↑	↓	93	11	31	
PKM2	Pyruvate kinase isozymes M1/M2	Metabolism; Energy pathways	Kinase activity	KPYM_HUMAN	58470	7.96	↑	↓	↓	91	15	34	[24] (↓)
PRDX6	Peroxiredoxin-6	Metabolism; Energy pathways	Peroxidase activity	PRDX6_HUMAN	25133	6	↓	↓	↑	227	14	69	[17] (↓) [16] (↑)
TKT	Transketolase	Metabolism; energy pathways	Catalytic activity	TKT_HUMAN	68519	7.58	↓	↓	↑	253	22	49	[13] (↑) [18] (↑)

Table 2 continued

Gene name	Protein name	Biological process	Molecular function	Uniprot access	MW	pI	Regulation control	Regulation 1 day	Regulation 3 days	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
CCT6A	T-complex protein 1 subunit zeta	Protein metabolism	Chaperone activity	TCPZ_HUMAN	58444	6.23	↑	↓	↓	69	11	22	
PDIA3*,#	Protein disulfide-isomerase A3	Protein metabolism	Isomerase activity	PDIA3_HUMAN	57146	5.98	↑	↓	↓	99	18	39	
EEF2	Elongation factor 2	Regulation of cell cycle	Transcription regulator activity	EF2_HUMAN	96246	6.41	↑	↑	↓	308	44	41	
ANXA1*,#	Annexin A1	Signal transduction; Cell communication	Calcium ion binding	ANXA1_HUMAN	38918	6.57	↑	↓	↑	226	14	50	
ANXA2*	Annexin A2	Signal transduction; cell communication	Calcium ion binding	ANXA2_HUMAN	38808	7.57	↓	↑	↑	215	20	49	
SYT17*	Synaptotagmin-17	Signal transduction; cell communication	Calcium ion binding	SYT17_HUMAN	54443	7.23	↑	↓	↓	67	14	28	

* Proteins that also were found differentially expressed in the acute treatment

Proteins that already were found differentially expressed after 24 h of treatment

Table 3 Differentially expressed proteins after chronic treatment with 10 μM clozapine

Gene name	Protein name	Biological process	Molecular function	Access	MW	pI	Regulation control	Regulation 8 h	Regulation 72 h	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
TXNDC12	Thioredoxin domain-containing protein 12	Protein metabolism	Catalytic activity	TXD12_HUMAN	19365	5.24	↑	↓	↑	63	6	39	
ANXA2	Annexin A2	Signal transduction; cell communication	Calcium ion binding	ANXA2_HUMAN	38808	7.57	↓	↑	↑	215	20	49	
PRKCSH	Glucosidase 2 subunit beta	Metabolism; energy pathways	Glucosidase activity	GLU2B_HUMAN	60357	4.33	↑	↓	↓	112	16	35	
PKM2	**Pyruvate kinase isozymes M1/M2**	**Metabolism; energy pathways**	**Kinase activity**	**KPYM_HUMAN**	**58470**	**7.96**	↓	↑	↑	**203**	**27**	**58**	
ACTG1	**Actin, cytoplasmic 2**	**Cell growth and/or maintenance**	**Structural constituent of cytoskeleton**	**ACTG_HUMAN**	**42108**	**5.31**	↓	↑	↓	**117**	**26**	**69**	[13] (↓) [17] (↑)
ACTB	**Actin, cytoplasmic 1**	**Cell growth and/or maintenance**	**Structural constituent of cytoskeleton**	**ACTB_HUMAN**	**42052**	**5.29**	↓	↑	↓	**106**	**24**	**64**	[17] (↑)
STMN1	**Stathmin**	**Cell growth and/or maintenance; signal transduction**	**Signal transducer activity**	**STMN1_HUMAN**	**17292**	**5.76**	↓	↓	↑	**58**	**7**	**48**	
HSPA5A	**78 kDa glucose-regulated protein**	**Protein metabolism**	**Chaperone activity**	**GRP78_HUMAN**	**72402**	**5.07**	↓	↑	↑	**179**	**29**	**48**	

Bold cells represent proteins that were found differentially expressed also in the acute treatment with 50 μM clozapine

Table 4 Differentially expressed proteins after chronic treatment with 10 μM MK-801 and 10 μM clozapine, administered at the same time

Gene name	Protein name	Biological process	Molecular function	Access	MW	PI	Regulation in control	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
HSP90AA1	Heat shock protein HSP 90-alpha	Protein metabolism	Chaperone activity	HS90A_HUMAN	85006	4.9	↓	177	33	50	
HSP90B1	Endoplasmin	Protein metabolism	Heat shock protein activity	ENPL_HUMAN	92696	4.8	↓	248	25	30	
ACTG1	Actin, cytoplasmic 2	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTG_HUMAN	42108	5.3	↓	117	26	69	[13] (↓) [17] (↑)
ACTB	Actin, cytoplasmic 1	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTB_HUMAN	42052	5.3	↓	106	24	64	[17] (↑)
PRKCSH	Glucosidase 2 subunit beta	Metabolism; Energy pathways	Glucosidase activity	GLU2B_HUMAN	60357	4.3	↓	112	16	35	

Table 5 Differentially expressed proteins after chronic treatment with 10 μM MK-801 and addition of 10 μM clozapine 8 h later

Gene name	Protein name	Biological process	Molecular function	Access	MW	pI	Regulation in control	MASCOT score	ID peptides	Coverage (%)	Previously found in differentially expressed in SCZ human brain tissue
HSP90AA1	Heat shock protein HSP 90-alpha	Protein metabolism	Chaperone activity	HS90A_HUMAN	85006	4.9	↓	177	33	50	
ACTG1	Actin, cytoplasmic 2	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTG_HUMAN	42108	5.3	↓	117	26	69	[13] (↓) [17] (↑)
ACTB	Actin, cytoplasmic 1	Cell growth and/or maintenance	Structural constituent of cytoskeleton	ACTB_HUMAN	42052	5.3	↓	106	24	64	[17] (↑)

Regarding some potential limitations of our study (1) although our results are encouraging, our study may not necessarily reflect the exact physiological process, as any other study with cell lines in vitro which tries to represent in vivo conditions; (2) as presented in Tables 1, 2, 3, 4, and 5, some of the differentially expressed proteins here presented were also found differentially expressed in SCZ human brain tissue but in different directions, what is expected and has been observed previously. Different cell types present peculiar protein expression, and this expression is differently modulated depending on how they are connected.

Concluding remarks

MK-801 treatment of cultured astrocytes showed similarities with the proteome analyses of MK-801-treated rats, but more interestingly with the differential expression of proteins found in SCZ human brain tissue. This demonstrates not only the probable usefulness of MK-801 in understanding SCZ molecular mechanisms in vivo and perhaps in vitro but also how cultured astrocytes may be contextualized in SCZ research. Moreover, clozapine treatment was effective in reversing differences observed in astrocytes after treatment with MK-801 as previously observed in behavioral experiments in rodents.

Although in vitro investigations may seem a bit far from the ideal while investigating psychiatric disorders, our results could also warrant further experiments such as the expression patterns in vitro of NMDA receptors subunits in order to provide detailed information about MK-801 action; the response of other medicaments over astrocytic proteome; and the stimulation of other cell lines with MK-801—or any other receptors agonists/antagonists—as well as primary cell cultures. The proposed experiments might shed light in the comprehension of the involved biochemical pathways providing useful insights for SCZ studies.

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