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STATHMIN REDUCTION AND CYTOSKELETON REARRANGEMENT IN RAT NUCLEUS ACCUMBENS IN RESPONSE TO CLOZAPINE AND RISPERIDONE TREATMENT – COMPARATIVE PROTEOMIC STUDY

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Abstract—The complex network of anatomical connections of the nucleus accumbens (NAc) makes it an interface responsible for the selection and integration of cognitive and affective information to modulate appetitive or aversively motivated behaviour. There is evidence for NAc dysfunction in schizophrenia. NAc also seems to be important for antipsychotic drug action, but the biochemical characteristics of drug-induced alterations within NAc remain incompletely characterized. In this study, a comprehensive proteomic analysis was performed to describe the differences in the mechanisms of action of clozapine (CLO) and risperidone (RIS) in the rat NAc. Both antipsychotics influenced the level of microtubule-regulating proteins, i.e., stathmin, and proteins of the collapsin response mediator protein family (CRMPs), and only CLO affected NAD-dependent protein deacetylase sirtuin-2 and septin 6. Both antipsychotics induced changes in levels of other cytoskeleton-related proteins. CLO exclusively up-regulated proteins involved in neuroprotection, such as glutathione synthetase, heat-shock 70-kDa protein 8 and mitochondrial heat-shock protein 75. RIS tuned cell function by changing the pattern of post-translational modifications of some proteins: it down-regulated the phosphorylated forms of stathmin and dopamine and the cyclic AMP-regulated phosphoprotein (DARPP-32) isoform but up-regulated cyclin-dependent kinase 5 (Cdk5). RIS modulated the level and phosphorylation state of synaptic proteins: synapsin-2, synaptotagmin-1 and adaptor-related protein-2 (AP-2) complex.

Key words: nucleus accumbens, clozapine, risperidone, 2D-DIGE, stathmin, glutathione synthetase.

INTRODUCTION

One of the structures of the mesolimbic dopamine (DA) pathway targeted by antipsychotic drugs is the nucleus accumbens (NAc). The NAc constitutes a main part of the ventral striatum (VS) and plays a significant role in cognitive processes related to reward, motivation and decision making (Carlezon and Thomas, 2009; Sesack and Grace, 2010; Durieux et al., 2011; Grueter et al., 2013; Gale et al., 2014; Floresco, 2015).

There are convergent findings indicated by post mortem studies of schizophrenic brains concerning a significant reduction of neuron number in the NAc (Jarskog et al., 2005). Volumetric studies of NAc in schizophrenia gave inconsistent results showing increased (Lauer et al., 2001), increased left side only (Spoletini et al., 2011), or decreased (Kreczmanski et al., 2007) or no significant differences (Haukvik et al., 2010; Womer et al., 2014) in NAc volume. However, many functional magnetic resonance imaging (fMRI) studies showed NAc/VS functional abnormality. Compared to healthy controls, unmedicated schizophrenic patients showed reduced VS activation during the presentation of reward-denoting cues that contributes to the formation of negative symptoms (Juckel et al., 2006). Moreover, deficiency in VS activation during reward anticipation is related to motivational deficits in schizophrenia (Esslinger et al., 2012). However, the inappropriately

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Abbreviations: 2DE, two-dimensional gel electrophoresis; ACN, acetonitrile; AP-2, adaptor-related protein-2; AP2b1, AP-2 complex subunit beta; Cdk5, cyclin-dependent kinase 5; CLO, clozapine; CRMPs, collapsin response mediator protein family, dihydropyrimidinase-related proteins; DA, dopamine; DARPP-32, dopamine and cyclic AMP-regulated phosphoprotein, protein phosphatase 1 regulatory subunit 1B; DIA, differential in-gel analysis; DIGE, differential in-gel electrophoresis; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; emPAI, exponentially modified protein abundance index; EPS, extrapyramidal symptoms; FGAs, first-generation antipsychotics; GSH, glutathione; GSS, glutathione synthetase; Hsp75, Heat-shock protein 75 kDa, mitochondrial; HSPA8, heat-shock 70-kDa protein 8, heat-shock cognate 71-kDa protein; IPA, Ingenuity Pathway Analysis; LASP-1, Lim and SH3 domain protein; LTP, long-term potentiation; MS, mass spectrometry; MSNs, medium spiny neurons; MT, microtubule; NAc, nucleus accumbens; NF-L, intermediate filament protein L, neurofilament light polypeptide; NUBP2, cytosolic Fe–S cluster assembly factor; PFC, prefrontal cortex; PKA, protein kinase A; Rheb, Ras homolog enriched in brain, GTP-binding protein Rheb; RIS, risperidone; SEPT6, septin 6; SGAs, second-generation antipsychotics; SIRT2, NAD-dependent protein deacetylase sirtuin-2; Stmn1, stathmin; STRAP, serine-threonine kinase receptor-associated protein; SynII, synapsin-2; Syt1, synaptotagmin-1; VS, ventral striatum.

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strong activation in the VS observed in schizophrenia patients in response to neutral stimuli leads to positive symptoms and an aberrant pattern of learning (Jensen et al., 2008).

Among many antipsychotic medicines, two seem to exhibit a remarkable clinical recommendation. Risperidone (RIS) is among the most frequently used second-generation antipsychotics (SGAs, atypical) in the treatment of chronic schizophrenia. At a standard therapeutic dose, it causes milder extrapyramidal symptoms (EPS) than first-generation antipsychotics (FGAs, typical). RIS can also be used for the treatment of autism, obsessive–compulsive disorder and agitation in dementia (Werner and Coveñas, 2014). Clozapine (CLO) is the only evidence-based efficient antipsychotic for the treatment of refractory schizophrenia (Buckley and Gaughran, 2014). It is most likely also superior in reducing aggression and suicidality (Melzer et al., 2003; Zarzar and McEvoy, 2013) and all-cause mortality (Tiihonen et al., 2009). In meta-analysis trials concerning treatment-resistant schizophrenia, CLO appeared to be better than FGAs in terms of psychopathology reduction and milder EPS (Miyamoto et al., 2012).

Generally, SGAs exhibit higher binding potency to serotonin receptors, especially in terms of antagonism to 5-HT2A, than to D2 receptors. Although both CLO and RIS are classified as SGAs, they differ in their pharmacological profiles (Ellenbroek and Cesura, 2015). The complex pattern of their mechanism of action becomes extremely tangled if one also considers mutual interactions between neurotransmitter systems. The role of NAc in the mechanism of action of antipsychotic drugs remains unclear, and its priority as the brain structure responsible for their therapeutic efficiency remains under debate. Therefore, further studies allowing more detailed characterization of the biochemical changes exerted by antipsychotics are required.

Proteomic studies could provide a broader and more holistic picture than other approaches. Although there are many proteomic studies concerning schizophrenia (see Table 2), only a few proteomic reports are related to the mechanism of antipsychotic action in brain tissue. Investigations conducted in this area have concerned the influence of chronic RIS treatment (2.1 mg/kg, 4 weeks) on striatum proteome (O'Brien et al., 2006); the impact of CLO (45 mg/kg) and chlorpromazine action (30 days) on the hippocampus protein profile (La et al., 2006); the effect of CLO (20 mg/kg), haloperidol and olanzapine (4 weeks) on the rat hippocampus (Chen and Chen, 2007); or – in another study – the effect of CLO (45 mg/kg), chlorpromazine and quetiapine (34 days) on the mitochondrial subproteome of the hippocampus and cerebral cortex (Ji et al., 2009). O'Brien's work provided a broader picture of proteomic changes, as 30 differential proteins were reported herein. In other studies, usually 1 or 2 but up to six alterations induced by CLO have been found. These results are difficult to compare, as they reflect too small a part of the changes in the protein profile; moreover, protein alterations seem to be tissue-dependent. It should be emphasized that there are no proteomics studies concerning the mechanism of action of antipsychotics in the NAc.

In vitro studies were also performed to determine the effects of CLO (7.65 μM), RIS (8 μM) and haloperidol (7 days) on the protein profile of C6 glioma cells (the same four differential proteins were found for both CLO and RIS) (Chen, 2013) and the impact of RIS (3 μM) and haloperidol on neural stem cells (60 and 14 differential proteins were identified for RIS after 24 and 96 h treatment, respectively) (Kashem et al., 2009; Ahmed et al., 2012).

All of the above studies were performed using two-dimensional electrophoresis (2DE) with Coomassie Blue, colloidal Coomassie Blue or silver staining, which have limited sensitivity or linearity. A 2DE approach with SYPRO Ruby fluorescent staining was employed to study the influence of CLO (20 mg/kg) and haloperidol (8 days treatment) on the cortex (two differential proteins for CLO) and thalamus (one differential protein for CLO) proteomes in a pharmacological model of schizophrenia (NMDA receptor antagonism) (Paulson et al., 2007). However, in present study, the two-dimensional differential in-gel electrophoresis (2D-DIGE) technique was applied as the most reproducible among gel-based proteomic approaches.

A mass spectrometry (MS)-based proteomic study of the influence of a very low dose of RIS (0.045 mg/kg, treatment of adolescent animals – postnatal days 34–47) on the adult rat prefrontal cortex (PFC) revealed 492 differential proteins implicated in, e.g., mitochondrial function and cytoskeleton structure (Farrelly et al., 2014).

Although CLO remains a gold standard in schizophrenia treatment, the source of its biochemical uniqueness remains unclear. Considering the extremely complex pharmacological profile of RIS and, in particular, CLO, the mutual interactions among many neurotransmitter systems within NAc as well as the influence of many brain areas on NAc and the net effect of the action of antipsychotics on NAc is beyond prediction. Thus, in this study, we characterized the alterations in the NAc proteome induced by RIS and CLO treatment of healthy rats.

EXPERIMENTAL PROCEDURES

Reagents

CLO, RIS, dimethylformamide (DMF), EDTA, L-lysine, Tween 20 were purchased from Sigma, St. Louis, MO, USA. Immobilized pH gradient strips 3–10 NL 24 cm, IPG Buffer pH 3–10 NL, Drystrip Cover Fluid, PlusOneBind-Silane, CyDye DIGE Fluor minimal labeling kit were acquired from GE Healthcare Life Sciences (Uppsala, Sweden). Acrylamide, bis-acrylamide, CHAPS (3-[(3-cholamidopropyl)dim-ethylammonio]-1-propanesulfonate), urea, thiourea, bromophenol blue, dithiothreitol (DTT), iodoacetamide, sodium dodecyl sulphate (SDS), glycine, agarose, Tris were purchased from BioShop Canada Inc. Ethanol, chloroform, methanol, glycerol, acetic acid were purchased from POCh (Gliwice, Poland). Ammonium bicarbonate and Coommassie Brillant Blue G-250 were from Fluka. Sequencing Grade Modified Trypsin was purchased from Biocentrum