Possible involvement of post-dopamine D\textsubscript{2} receptor signalling components in the pathophysiology of schizophrenia

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Abstract

Par-4 has been suggested to mediate dopamine neurotransmission. Dopamine D\textsubscript{2} receptor (DRD2) activation induces a signalling complex of AKT1, PP2A and \(\beta\)-arrestin2 which dephosphorylates/inactivates AKT1 thereby activating GSK-3\(\beta\), transducing dopamine-dependent behaviour. DRD2 activation also results in down-regulation of PKA activity. Among other substrates PKA phosphorylates GSK-3\(\beta\). Prolonged DRD2 activation leads to its ‘desensitization’ which involves GRKs and \(\beta\)-arrestins. \(\beta\)-arrestin1 binds to phosphorylated receptors preventing further G-protein stimulation. This study examined whether Par-4, \(\beta\)-arrestin1, AKT1 and GSK-3\(\beta\) are involved in the pathophysiology of schizophrenia. Lymphocytes obtained from schizophrenia and bipolar patients and healthy controls recruited from the Beer-Sheva Mental Health Center were transformed by Epstein–Barr virus (EBV) into lymphocyte-derived cell lines (LDCL). Post-mortem brain samples were obtained from the Rebecca L. Cooper Brain Bank, Parkville, Australia. The study was approved by the IRB committees of Beer-Sheva, Israel and Parkville, Australia. Levels of the specific proteins were assayed by Western blotting. \(\beta\)-arrestin1 protein levels were significantly \(\sim 2\)-fold increased in LDCL from schizophrenia patients while Par-4 protein levels were unaltered. A 63\% significant decrease was found in frontal cortex phospho-Ser9-GSK-3\(\beta\) protein levels in schizophrenia but not in those of AKT1, Par-4 or \(\beta\)-arrestin1. Elevated \(\beta\)-arrestin1 protein levels in LDCL and decreased phospho-Ser9-GSK-3\(\beta\) protein levels in post-mortem frontal cortex of schizophrenia patients vs. control groups support the possible involvement of these proteins in the pathophysiology of schizophrenia. However, since we did not find differences in \(\beta\)-arrestin1, AKT1 and Par-4 protein levels in post-mortem frontal cortex of schizophrenia patients and although GSK-3\(\beta\) participates in other signalling cascades we can not rule out the possibility that the differences found reflect deviation in DRD2 signalling.

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Introduction

Dopamine D\textsubscript{2} receptors (DRD2), which are targeted by all antipsychotic drugs, belong to the G-protein coupled receptor (GPCR) family. DRD2 signals have been suggested to be mediated by prostate apoptosis response 4 (Par-4), \(\beta\)-Arrestins, AKT [also designated protein kinase B (PKB)]1 and glycogen synthase kinase (GSK)-3\(\beta\) (Figure 1). Activation of DRD2 leads to down-regulation of cyclic AMP (cAMP) production resulting in decreased phosphorylation of proteins including GSK-3\(\beta\) (Li et al., 2000). Activation of DRD2 may lead to the formation of a signalling complex composed of AKT1, protein phosphatase-2A (PP2A) and \(\beta\)-arrestin2, which dephosphorylates phosphothreonine-308 (Thr308) AKT1 residues resulting in AKT1 inactivation and a concomitant activation of GSK-3\(\beta\), affecting transduction processes thought to be involved in mediating dopamine-dependent behaviour (Beaulieu et al., 2004, 2005). Some post-mortem,
Desensitization

Elevate the level of the inactive Ser9 phosphorylated haloperidol and clozapine have been reported to inactivate, in antipsychotic drug action. Moreover, Li and colleagues have shown that atypical anti-inflammatory drugs target GSK-3 (2003) suggest the involvement of phosphorylation of Akt2 (Alimohamad et al., 2005). GSK-3 is a negative regulator of signalling cascades (e.g. DRD2, phosphatidylinositol-3-Kinase/AKT and Wnt) found to be altered in schizophrenia (Beaulieu et al., 2004; Cotter et al., 1998; Emamian et al., 2004; Papkoff and Aikawa, 2004; Szczepankiewicz et al., 2006). GSK-3 is highly abundant in the brain. A double-labelling fluorescent microscopy study showed that populations of neurons present alteration in GSK-3 also expressed DRD2 (Alimohamad et al., 2005). GSK-3 is a negative regulator of signalling cascades (e.g. DRD2, phosphatidylinositol-3-Kinase/AKT and Wnt) found to be altered in schizophrenia (Beaulieu et al., 2004; Cotter et al., 1998; Emamian et al., 2004; Papkoff and Aikawa, 1998). Since the levels of kinases, including GSK-3, are regulated not only at the transcription level but also by phosphorylation, several recent studies (Beaulieu et al., 2004, 2005; Emamian et al., 2004; Svenningsson et al., 2003) suggest the involvement of phosphorylation of GSK-3β on its Ser9 residue, resulting in the enzyme’s inactivation, in antipsychotic drug action. Moreover, Li and colleagues have shown that atypical antipsychotics inhibit GSK-3 activity (Li et al., 2007), and haloperidol and clozapine have been reported to elevate the level of the inactive Ser9 phosphorylated form of GSK-3β (Kang et al., 2004; Kozlovsky et al., 2006). All these data support the hypothesis that antipsychotic drugs target GSK-3.

Given the potential for the involvement in antipsychotic drug action, it is significant that a study of dopamine transporter (DAT) knock-out mice by Beaulieu et al. (2004) which showed that increased dopamine neurotransmission results in inactivation of AKT1 and a concomitant activation of GSK-3β, has led to the suggestion of an association between schizophrenia and an alteration in the AKT1/GSK-3 signalling cascade (Beaulieu et al., 2004). The same study showed that pharmacological or genetic inhibition of GSK-3 results in reduced hyperlocomotion in the DAT mouse, a dopamine-dependent behaviour (Beaulieu et al., 2004). Moreover, the study of Emamian et al. (2004), using peripheral lymphocytes and post-mortem brain from schizophrenia patients as well as rodents treated with antipsychotics, also presented data that support the notion that alteration in the AKT1/GSK-3 signalling cascade contributes to the pathophysiology of schizophrenia. These authors suggested that AKT1 could be a potential susceptibility gene for schizophrenia. Additional studies show reduction in AKT1 content and activity in post-mortem brain from schizophrenia patients compared with healthy controls (Zhao et al., 2006) and a genetic contribution of AKT1 polymorphisms to schizophrenia (Bajestan et al., 2006; Ikeda et al., 2004; Schwab et al., 2005). A recent study suggests that deficiency of AKT1 affects neuronal architecture and modulates prefrontal cortex functioning (Lai et al., 2006). Given the recognized role of cortical dysfunction in the pathology of schizophrenia (Harrison and Weinberger, 2005) it would seem likely that changes in AKT1 in this region of the CNS could contribute to the genesis of symptoms associated with the disorder.

Similarly to other G-protein coupled receptors prolonged activation of DRD2 results in ‘desensitization’ (Kim et al., 2001; Oakley et al., 2001). The cascade of events of ‘desensitization’ has been suggested to involve two families of proteins – the GRKs and the β-arrestins (Gainetdinov et al., 2004). β-arrestins have been shown in COS7 and HEK-293 cells to bind to phosphorylated receptors and prevent further stimulation of G proteins and downstream signalling pathways (Kim et al., 2001). β-arrestins also act as signal transducers of their own, activating the pathways of tyrosine kinases (TKs), mitogen-activated protein kinase (MAPK), phosphatidyl inositol-3 kinase (PI3K), AKTI and nuclear factor kappa B (NFκB) (Lefkowitz and Shenoy, 2005). Hypothetically, β-arrestins may thus be involved in dopaminergic transmission via AKTI.
Par-4, a pro-apoptotic factor (Sells et al., 1994), is expressed in neurons. Previous studies in animal models implied its possible involvement in neuronal death in Parkinson’s disease, Alzheimer’s disease, stroke and amyotrophic lateral sclerosis (Culmsee et al., 2001; Duan et al., 1999; Guo et al., 1998; Pedersen et al., 2000). A recent study demonstrated that Par-4 may play a role in mediating DA neurotransmission at striatal synapses and that mice lacking the domain of Par-4 interacting with DRD2 show increased depression-like behaviour (Park et al., 2005).

We hypothesized that Par-4, β-arrestin1, AKT1 and phospho-Ser9-GSK-3β are involved in the pathophysiology of schizophrenia, contributing to the altered dopamine transmission proposed to underlie the pathophysiology of this disorder. To test this hypothesis we measured their protein levels in LDCL and in post-mortem frontal cortex of schizophrenia patients and control subjects. LDCL were used as a cell model devoid of confounding factors such as psychotropic drug treatment.

Methods

LDCL

EBV-transformed lymphoblastoid cell lines were established and grown as previously described (Bennett et al., 1991; Ebstein et al., 1990). Cell lines were established from three diagnostic groups of subjects. Control subjects [11 male, 11 female; average age 38.2 ± 2.2 yr (± S.E.M.), range 22–50 yr] with no history of psychiatric illness were recruited from the Beer-Sheva area. Non-hospitalized bipolar patients [9 males, 8 females; average age 46.6 ± 2.5 yr (± S.E.M.), range 23–62 yr] were recruited from the Mood Disorder Clinic of the Beer-Sheva Mental Health Center and schizophrenia patients [9 males, 8 females; average age 40.1 ± 2.5 yr (± S.E.M.), range 22–61 yr] were hospitalized patients from the Beer-Sheva Mental Health Center. All patients were diagnosed according to DSM-IV criteria. The study was approved by the Beer-Sheva hospital Helsinki committee (IRB). All patients gave written informed consent. The investigators who carried out the assays (A.S. and S.G.) were blind to the code.

Cell extract preparation for the measurement of β-arrestin1 and Par-4 protein levels

The LDCL were first washed three times with phosphate-buffered saline (PBS) centrifugation at 2000 g for 5 min at 4 °C. Sediment of washed cells from each subject was sonicated for 10 s, 4 °C at 50% power capacity (Heat System Ultrasonic, Newtown, CT, USA) in 250–350 μl lysis buffer [50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM Na-glycerophosphate, 5 mM Na-pyrophosphate, 1 mM Na-orthovanadate, 1% (v/v) 2-mercaptoethanol, 10 µg/ml of: leupeptine, aprotinin and pepstatin, 10 mM benzamidine hydrochloride and 1 mM PMSF]. Triton X-100 [0.1% (v/v)] was added and cell lysates incubated in an orbital shaker for 20 min at 4 °C. After centrifugation at 10 000 g for 15 min at 4 °C the supernatant was collected and protein concentration determined (Bradford, Bio-Rad, Hercules, CA, USA). The lysates were used for Western-blot analysis. This study was approved by the IRB committees of Parkville and Beer-Sheva.

Post-mortem frontal cortex samples for β-Arrestin1, AKT1, Par-4 and phospho-Ser9-GSK-3β protein levels

Post-mortem brain samples from 15 schizophrenia patients and 15 matched normal controls were obtained from the Victorian Brain Tissue Repository at the Mental Health Research Institute of Victoria, Melbourne, Australia. Samples studied were of frontal cortex [Brodmann’s Area (BA) 9]. Table 1 summarizes the demographic data of the subjects.

Cronic haloperidol treatment of rats

Adult 200–250 g Sprague–Dawley rats (Harlan, Jerusalem) were housed in a rat colony room with constant temperature (22–23 °C) and a 12 h light/dark cycles. Food and water were provided ad libitum and contained drugs as described below. The Ben-Gurion University Medical School Review Committee for the use of animals approved the experimental protocol and the procedures were in compliance with the

<table>
<thead>
<tr>
<th>Subjects (M/F)</th>
<th>Healthy controls</th>
<th>Schizophrenia patients</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>47 ± 15</td>
<td>47 ± 15</td>
</tr>
<tr>
<td>pH</td>
<td>6 ± 0.2</td>
<td>6 ± 0.2</td>
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<tr>
<td>PMI (h)</td>
<td>42 ± 16</td>
<td>44 ± 13</td>
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<tr>
<td>DOI (yr)</td>
<td>–</td>
<td>17 ± 12</td>
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M, Male; F, female; PMI, post-mortem interval; DOI, duration of illness.

All schizophrenia patients were medicated.

Data are expressed as mean ± S.D.

Table 1. Demographic data of the subjects of the Australian Brain Bank

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National Institute of Health Guide for Care and Use of Laboratory Animals. Rats were injected with 10 mg/kg haloperidol (Janssen-Cilag, Beerse, Belgium) i.p. daily for 21 d as a solution in 0.2% citric acid. Control rats were injected with citric acid only. Haloperidol-treated rats were sacrificed 24 h after the last injection. After decapitation brains were removed, frontal cortex specimens dissected and frozen at −80 °C until processed. The drug dose administrated was based upon previous studies in which it has been shown that this regime results in rodent blood levels within the therapeutic range in patients (Jardemark et al., 2000; Shapiro et al., 2000).

**Western blot analysis of AKT1, phospho-Ser9-GSK-3β, Par-4 and β-arrestin1**

Sodiumdodecyl sulphate–polyacrylamide gel electrophoretic separation (SDS–PAGE) and immunoblotting of β-arrestin1, AKT1, Par-4 and phospho-Ser9-GSK-3β were performed using a previously described procedure (Nadri et al., 2004) with modifications. Aliquots of 10–20 µg total protein, within the linear range of quantitative detection were separated (10% gel, 180 V), blotted, and probed overnight at 4 °C with diluted (1:100) monoclonal anti mouse β-arrestin1 antibodies (Transduction Labs, Franklin Lakes, NJ, USA) (Figure 2a), or diluted (1:250) monoclonal anti mouse AKT1 antibodies (Cell Signaling, Danvers, MA, USA) (Figure 2b), or diluted (1:250) monoclonal anti rabbit Par-4 antibodies (Transduction Labs) (Figure 2c), or diluted (1:100) monoclonal anti rabbit phospho-Ser9-GSK-3β antibodies (Transduction Labs) (Figure 2d). Bands were detected with Chemiluminescence Western-blot Detection kit (Amersham, Oakville, ON, Canada). To minimize the effect of interblot variability, a calibration standard curve of 10 µg, 20 µg and 40 µg total protein of a given brain extract was run in each gel. Densities of the immunoreactive bands were quantified using AIDA-2D image analysis system (Dinco and Rehenium, Jerusalem, Israel).

Statistical analysis was done using STATISTICA 7 software (StatSoft Inc., Tulsa, OK, USA). Student’s t test or ANOVA were carried out as applicable following Levene’s test showing homogenous distribution for all studied proteins. Where indicated, outliers were identified using Grubb’s test (Stefansky, 1972).

**Results**

**β-arrestin1 protein levels**

**LDCL**

A significant increase in LDCL β-arrestin1 protein levels in 12 schizophrenia patients [3.8 ± 0.7 arbitrary units (AU) (± S.E.M.)] compared with 12 bipolar patients (1.7 ± 0.7 AU) and 12 control subjects (1.6 ± 0.7 AU) ($F_{2,33} = 3.41, p = 0.045$) with an LSD post-hoc comparison revealing a significant increase in schizophrenia vs. bipolar patients ($p = 0.037$) and vs. control subjects ($p = 0.025$), but no difference between bipolar patients and control subjects ($p = 0.85$) (Figure 3). There was no correlation between β-arrestin1 protein levels and age ($r = 0.21, p = 0.2$) and no effect of gender (Student’s t test: $t = 0.44, p = 0.66$).

**Post-mortem frontal cortex**

There was no difference between β-arrestin1 protein levels in post-mortem frontal cortex of 15 schizophrenia patients (0.65 ± 0.08 AU) compared with 14 control subjects (0.62 ± 0.05 AU) ($t = 0.36, p = 0.72$). One control sample showed β-arrestin1 band that was below the detection limit of our densitometry system. β-arrestin1 protein levels did not correlate with post-mortem interval (PMI), pH of the tissue, duration of illness (DOI) or age (Table 2) and did not differ between males and females [0.67 ± 0.053 AU, $n = 22$ vs. 0.55 ± 0.094 AU, $n = 7$, respectively, $t = 1.08, p = 0.29$].

**Frontal cortex of haloperidol-treated rats**

To find out whether the discrepancy between the finding in LDCL and post-mortem frontal cortex stems...
from antipsychotic treatment of the schizophrenia patients from whom post-mortem brain specimens were obtained rats were treated with the upper range of haloperidol dose and frontal cortex β-arrestin1 protein levels measured. There was no difference between β-arrestin1 protein levels in the frontal cortex of seven haloperidol-treated rats (1.0 ± 0.25 AU) compared with eight control rats (0.8 ± 0.24 AU) (t = 0.64, p = 0.53).

LDCL and post-mortem frontal cortex Par-4 protein levels

There were no differences in Par-4 protein levels in LDCL from 17 schizophrenia patients (6.0 ± 0.7 AU) vs. 17 bipolar patients (6.7 ± 0.74 AU) and 22 control subjects (6.8 ± 0.76 AU) (F₁,₁₃ = 0.33, p = 0.72) or in post-mortem frontal cortex of 15 schizophrenia patients (2.5 ± 0.31 AU) vs. 15 control subjects (2.3 ± 0.35 AU) (t = 0.42, p = 0.68). Par-4 protein levels did not correlate with PMI, pH of the tissue, DOI or age (Table 2) and did not differ between males and females [2.55 ± 0.25 AU, n = 22 vs. 1.72 ± 0.5 AU, n = 6, respectively; t = 1.52, p = 0.14].

Post-mortem frontal cortex phospho-Ser9-GSK-3β protein levels

Grubbs’ test [maximum normalized residual test (Stefansky, 1972)] identified two outlier results in the schizophrenia patients’ group. Omitting these samples from the analysis resulted in 63% significant decrease in phospho-Ser9-GSK-3β protein levels in post-mortem frontal cortex of 13 schizophrenia patients (0.6 ± 0.13 AU) compared with 15 control subjects (1.63 ± 0.34 AU) (t = 2.66, p = 0.013) (Figure 4). Phospho-Ser9-GSK-3β protein levels did not correlate with PMI, pH of the tissue, DOI or age (Table 2) and did not differ between males and females [1.3 ± 0.28 AU, n = 20 vs. 0.8 ± 0.2 AU, n = 8, respectively; t = 1.03, p = 0.3].

Post-mortem frontal cortex AKT1 protein levels

There was no difference between AKT1 protein levels in post-mortem frontal cortex of 14 schizophrenia patients (0.46 ± 0.05 AU) compared with 14 control subjects (0.5 ± 0.03 AU) (t = 0.57, p = 0.57). One sample from each diagnostic group showed AKT1 bands that were below detection limit of the densitometry system. AKT1 protein levels did not correlate with PMI, pH of the tissue, DOI or age (Table 2) and did not differ between males and females [0.46 ± 0.04 AU, n = 22 vs. 0.53 ± 0.09 AU, n = 8, respectively; t = −0.86, p = 0.39].

Discussion

The notion that dopamine D₂ receptor hyperactivity underlies the pathophysiology of schizophrenia is still questionable due, among others, to controversial findings regarding the density of D₂ dopamine receptors in the brain of schizophrenia patients (Davis et al., 1991; Laruelle, 1998; Seeman and Kapur, 2000). A meta-analysis of 13 in-vivo studies (Laruelle, 1998) which revealed 12% elevated D₂ dopamine receptor density in drug-naive and in drug-free schizophrenia patients does support the dopamine D₂ receptor hyperactivity hypothesis. Interestingly, a recent animal study demonstrated correlation between increased striatal DRD2 density and impaired prefrontal cortex cognition (Kellendonk et al., 2006). The present study...
examined the possible involvement of four key proteins in the dopamine D2 receptor signal transduction pathway, Par-4, AKT1, GSK-3β and β-arrestin1 in the pathophysiology of schizophrenia, by measuring their protein levels in post-mortem brain and in LDCL from schizophrenia patients vs. normal control subjects.

β-arrestin1 possibly regulates ‘desensitization’ of D1 and D2 dopamine receptors (Kim et al., 2001; Oakley et al., 2001). The nearly 2-fold significantly elevated LDCL β-arrestin1 protein levels in schizophrenia patients may reflect such D2 receptor desensitization due to hyperdopaminergic activity hypothesized to occur in schizophrenia. Genetic findings support the possible involvement of β-arrestin1 in the aetiology of schizophrenia. Its gene is located on the long arm of chromosome 11 (11q13) (Calabrese et al., 1994) near the locus of the dopamine D2 receptor gene (Grandy et al., 1989). This locus has been reported to be linked to schizophrenia (Lewis et al., 2003; Paunio et al., 2004). The lack of difference in β-arrestin1 protein levels in post-mortem frontal cortex of schizophrenia patients compared with control subjects vs. the increased levels in LDCL of patients may have been caused by demographic or environmental confounding factors affecting the post-mortem brain samples. However, no correlation between post-mortem frontal cortex β-arrestin1 protein levels and age, DOI, pH of the tissue and PMI was obtained, and there was no effect of gender. The fact that rat frontal cortex β-arrestin1 protein levels were not affected by chronic haloperidol treatment also does not favour the possibility that neuroleptic treatment of the patients normalized their brain β-arrestin1 protein levels. It may not be ruled out that lymphocytes from schizophrenia patients respond differently to the EBV transformation.

β-arrestin1 also acts as a signal transducer of its own possibly activating AKT1 (Lefkowitz and Shenoy, 2005). Activated AKT1 phosphorylates the Ser9 residue of GSK-3β and thereby inactivates it. Thus, elevated β-arrestin1 protein levels in schizophrenia would have been expected to lead to elevated phospho-Ser9-GSK-3β levels. Yet, the post-mortem brain results of the present study as well as of others (Emamian et al., 2004) show decreased rather than elevated phospho-Ser9-GSK-3β levels. This suggests that elevated LDCL β-arrestin1 protein levels do not reflect DRD2 desensitization. Interestingly, a similar discrepancy between a finding in LDCL vs. post-mortem brain has already been reported by our group concerning inositol monophosphatase activity in bipolar patients (Shaltiel et al., 2001). As in the present study no obvious confounding factor was found to account for the discrepancy.

Recent studies by Avissar and co-workers (Avissar et al., 2004; Matuzany-Ruban et al., 2005) of significantly reduced leukocyte β-arrestin1 mRNA and protein levels in unipolar depressed patients compared with control subjects indicate specificity of the present finding of elevated LDCL β-arrestin1 protein levels in schizophrenia. Further studies of β-arrestin1 protein levels in fresh lymphocytes from schizophrenia patients vs. control subjects need to be done to conclude whether β-arrestin1 protein levels could be used as a marker for schizophrenia.

The AKT1/GSK-3 pathway has recently been suggested to mediate dopamine neurotransmission. Mice lacking the dopamine transporter exhibit inactivation of AKT1 (as measured by reduced phospho-Thr308-AKT1 levels) and concomitant activation of GSK-3β (reduced phospho-Ser9-GSK-3β levels) in the striatum. These biochemical changes in the DAT null mice were reversed by inhibition of dopamine synthesis (Beaulieu et al., 2004). Several groups, including ours, reported differences in these proteins in post-mortem brain in schizophrenia. Frontal cortex and hippocampal AKT1 and phospho-Ser9-GSK-3β levels (Emamian et al., 2004), and frontal cortex (Beasley et al., 2001; Kozlovsy et al., 2000) and hippocampal (Nadri et al., 2004) total GSK-3β protein levels were found reduced. In the present study, using a different post-mortem brain bank, we replicated the result of Emamian et al. (2004) by finding a significant increase in the biochemical indices of the AKT1/GSK-3 pathway in schizophrenia patients compared with normal controls.
63% decrease in phospho-Ser9-GSK-3β protein levels in the frontal cortex of schizophrenia patients. Yet, in contrast to the results of Emamian et al. (2004) and Zhao et al. (2006), we did not find any difference in AKT1 protein levels between schizophrenia patients and control subjects, a finding which is consistent with another study that also failed to replicate Emamian et al.’s finding of altered frontal cortex total AKT1 protein levels in schizophrenia (Ide et al., 2006). Since Ide et al. (2006) and the present study used the same source of anti-AKT1 antibodies while Emamian et al. (2004) and Zhao et al. (2006) used two other different sources it may not be ruled out that the discrepancy between the studies stems from the use of different antibodies. Absence of changes in the total protein levels of AKT1 does not preclude the possibility that AKT1 phosphorylation/activity might be changed in schizophrenia patients’ brain.

We have previously reported reduced total GSK-3 activity in post-mortem prefrontal cortex of schizophrenia patients (Kozlovsky et al., 2001). The present result of reduced phospho-Ser9-GSK-3β levels is seemingly opposite to our previous finding. Two possible argumentations may be suggested to reconcile between the two findings. In our GSK-3 activity measurement we do not discriminate between the activity contributed by each of the GSK-3 isoforms (α and β). Alternatively, since we found reduced total GSK-3β protein levels, both the Ser9 phosphorylated and the non-phosphorylated (the active) forms may be reduced in the patients.

In conclusion, our significant finding of elevated β-arrestin1 protein levels in LDCL from schizophrenia patients vs. bipolar patients and control subjects, and the 63% decrease in phospho-Ser9-GSK-3β protein levels in post-mortem frontal cortex of schizophrenia patients compared with control subjects support the possible involvement of these proteins in the pathophysiology of schizophrenia. However, since we did not find differences in β-arrestin1, AKT1 and Par-4 protein levels in post-mortem frontal cortex of schizophrenia patients, and although GSK-3β participates in other signalling cascades, we can not rule out the possibility that the differences found reflect deviation in the DRD2 signalling.

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Statement of Interest
None.

References


Paunio T, Tuulio-Henriksson A, Hickklalinna T, Perola M, Varilo T, Partonen T, Cannon TD, Lonnqvist J,


