Cyclin-dependent kinase-5 and p35/p25 activators in schizophrenia and major depression prefrontal cortex: basal contents and effects of psychotropic medications

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Abstract

Cyclin-dependent kinase-5 (CDK5) and p35/p25 activators, interacting with the exocytotic machinery (e.g. munc18-1 and syntaxin-1A), play critical roles in neurosecretion. The basal status of CDK5/p35/p25 and the effect of psychotropic drugs (detected in blood/urine samples) were investigated in post-mortem prefrontal cortex (PFC)/Brodmann’s area 9 of schizophrenia (SZ) and major depression (MD) subjects. In SZ (all subjects, n=24), CDK5 and p35, but not p25, were reduced (−28 to −58%) compared to controls. In SZ antipsychotic-free (n=12), activator p35 was decreased (−52%). In SZ antipsychotic-treated (n=12), marked reductions of CDK5 (−47%), p35 (−76%) and p25 (−36%) were quantified. In MD (n=13), including antidepressant-free/treated subgroups, CDK5, p35 and p25 were unaltered. In SZ (n=24), CDK5, p35 or p25 correlated with munc18-1a, but not with syntaxin-1A. The results demonstrate reduced p35 basal content and down-regulation of CDK5/p35/p25 by antipsychotics in SZ. The suggested CDK5/munc18-1a functional interaction may lead to dysregulated neurosecretion in SZ PFC.

Introduction

Cyclin-dependent kinase-5 (CDK5) is a proline-directed serine/threonine kinase essential for many cellular processes, which include synaptic plasticity (Angelo et al. 2006; Barnett & Bibb, 2011) and neurotransmitter release (Chergui et al. 2004; Kim & Ryan, 2010; Tomizawa et al. 2002). CDK5 activation requires the cofactor p35, a non-cyclin regulatory protein, and/or p25, a p35 active product (Kusakawa et al. 2000; Tsai et al. 1994). Over the past decade, the synaptic hypothesis of schizophrenia (SZ) and major depressive disorder has revealed dysfunctions of neuroplasticity (Eastwood, 2004; Nissen et al. 2010) and neurosecretion (Gil-Pisa et al. 2012; Johnson et al. 2008), which might involve the participation of CDK5 and activators. Thus, the pharmacological or genetic inhibition of CDK5 activity was shown to potentiate presynaptic function in neurons, indicating that this kinase normally acts as a strong suppressor of neurotransmitter release (Kim & Ryan, 2010). Roscovitine, a CDK5 inhibitor, was also shown to increase striatal dopamine release and to regulate glutamatergic transmission (Chergui et al. 2004), two neurotransmitter systems involved in the pathogenesis of SZ (Marek et al. 2010). The presynaptic proteins munc18-1 and syntaxin-1A are important factors of the exocytotic machinery that control vesicle fusion and neurosecretion (Han et al. 2010; Rickman & Duncan, 2010). CDK5/p35 was reported to bind and phosphorylate munc18, which can then regulate munc18 × syntaxin-1A interaction in nerve endings (Fletcher et al. 1999; Shuang et al. 1998). In a recent study, syntaxin-1A was found increased and munc18-1a unchanged in SZ prefrontal cortex (PFC), and both proteins were down-regulated by antipsychotic drug treatment (Gil-Pisa et al. 2012). In contrast, munc18-1 and syntaxin-1A were unaltered in PFC of major depression (MD) subjects, regardless of antidepressant drug treatment (Gil-Pisa et al. 2012). Given the regulatory role of CDK5 in neurosecretion and munc18 × syntaxin-1A interaction, this study investigated the basal status of CDK5 and activators p35/p25 in post-mortem PFC of well-characterized subjects with SZ or MD, as well as the effects of psychotropic medications. Possible interrelationships between
CDK5/p35/p25 and munc18-1a or syntaxin-1A were also assessed in the same brain samples. A preliminary account was presented at the 24th Congress of the European College of Neuropsychopharmacology (Ramos-Miguel et al. 2011).

Materials and method

**Subject selection and post-mortem brain samples**

Human brains were obtained at autopsies performed in the Instituto Vasco de Medicina Legal, Bilbao (Spain) and the Centre Universitaire Romand de Médecine Légale–Site Genève, University of Geneva (Switzerland), following the established legal and ethical procedures for post-mortem brain investigations. The study design was approved by the Ethical Committee of Clinical Investigation (CEIC, Spain) and developed in accordance with the guidelines of the University of the Balearic Islands. Caucasian subjects who had died of various causes were subjected to a retrospective search for previous psychiatric diagnoses (DSM-IV) and medications as described (Gil-Pisa et al. 2012; Urigüen et al. 2009). Healthy controls (no evidence of psychiatric or neurological disorder) and subjects with SZ or MD were included in the study. These SZ and MD subjects had been used in a previous study that revealed alterations of key proteins of the exocytotic machinery in PFC (Brodmann’s area 9; BA9) of SZ subjects (Gil-Pisa et al. 2012). Subjects with SZ or MD [psychotropic-free: SZ(−) or MD(−); psychotropic-treated: SZ(+) or MD(+)] were matched to control subjects for post-mortem interval (PMI), brain pH, gender and age at death (Table 1; see Gil-Pisa et al. 2012 for individual features including causes of death and blood/urine levels of psychotropic drugs, which are also reported in the present study as Supplementary Tables S1 and S2). The effect of PMI (range 3–102 h) on the content of CDK5, p35 and p25 revealed a rapid degradation of p35, but not of CDK5 and p25, in PFC/BA9 (Ferrer-Alco´n et al. 2003). A wide range of PMI (5–102 h) has been shown indicating good tissue quality).

**Western blot analysis and quantification of target proteins**

Preparation of PFC/BA9 samples and immunoblot analysis of target proteins was assessed as described (Garcia-Fuster et al. 2008; Gil-Pisa et al. 2012). Briefly, brain proteins (20 μg sample, previously boiled for 2 min) were resolved by standard gel electrophoresis and Western blot procedures. The primary antibodies against CDK5 (mouse monoclonal Ab-1, clone DC-17, dilution 1:5000; Lab Vision, USA) and p35/p25 (rabbit polyclonal

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<th>Table 1. Demographic data of control, SZ and MD post-mortem brains</th>
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<td><strong>Groups and subgroups</strong></td>
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<td>Control (n = 17)</td>
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<td>MD(+) (n = 9)</td>
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SZ, Schizophrenia; MD, major depression; F, female; M, male; PMI, post-mortem interval; SZ(−), antipsychotic-free; SZ(+), antipsychotic-treated; MD(−), antidepressant-free; MD(+), antidepressant-treated.

Data (age, PMI, pH) are mean ± s.e.m. values (n, number of subjects). Control subjects in both series mainly died of accidental or violent causes. All controls had negative blood toxicology. SZ and MD subjects mainly died of violent suicide: SZ (n = 22); MD (n = 12).

Antipsychotics in SZ(+) subjects at the time of death (blood/urine): clozapine; clozapine; haloperidol; olanzapine; quetiapine. Antidepressants in MD(+) subjects at the time of death (blood/urine): citalopram; duloxetine; fluoxetine; mirtazapine; venlafaxine.

Ab C-19, batch K0409, dilution 1:3000; Santa Cruz Biotechnology, USA) were characterized in brain tissue (Ferrer-Alco´n et al. 2003). As a control for sample loading and protein transfer, the membranes were stripped and re-probed with anti-β-actin (mouse monoclonal Ab, clone AC-15, dilution 1:10 000; Sigma-Aldrich, USA). Membrane-bound specific immunoreactivity was assessed using the appropriate secondary antibody, a chemiluminescence method and light-sensitive films, which were finally measured by densitometry (integrated optical density). Target proteins in the PFC/BA9 were quantified in pairs of subjects with SZ or MD (drug-free and drug-treated), and the respective matched controls, and the relative protein content was calculated (percent change) in relation to in-gel triplicate standards (100%, pool of 17 or 14 control samples for SZ and MD groups, respectively). The inclusion of a standardization sample resulting mean value of the target protein was used as a final estimate. The content of CDK5, p35 and p25 in the PFC/BA9 was further normalized to that of β-actin.

**Data analyses and statistics**

The data were analysed with GraphPad Prism™, version 4.0 (GraphPad Software, USA). Results are expressed as
mean ± S.E.M. values. The various sets of data (SZ and MD groups) did not include outliers (inspected with Grubb’s test) and they all passed Kolmogorov–Smirnov normality tests. Data were analysed using Student’s t test and/or one-way analysis of variance followed by Bonferroni’s post-hoc test for multiple comparisons between groups and subgroups of subjects. In SZ and MD subjects, CDK5, p35 and p25 were also analysed by pairs [i.e. control subject vs. SZ(−) or SZ(+) or control subject vs. MD(−) or MD(+) in the corresponding gel] using paired t tests to further avoid confounding variables, especially the effect of PMI (see Ferrer-Alco´n et al. 2003). Pearson’s correlation coefficients (r) were calculated to test for possible association between neurochemical findings (i.e. CDK5/p35/p25 with specific proteins of the neurosecretory machinery or with PMI, gender and age). When a correlation was found between a demographic variable and target protein, an analysis of covariance (ANCOVA) was performed to correct for the confounding variable. These ANCOVAs indicated that PMI and age did not influence the results (data not shown). Similarly, the gender of the subjects did not alter the results (data not shown). All tests were two-tailed and the significance was set to p < 0.05.

Results

CDK5 and activators p35/p25 in PFC/BA9 of antipsychotic-free and antipsychotic-treated SZ subjects

Western blot analyses of all SZ subjects (n = 24) showed that the contents of CDK5 and p35, but not p25, were significantly reduced in PFC (CDK5: −28 ± 9%, t = 2.41, p < 0.05; p35: −58 ± 7%, t = 4.60, p < 0.001; p25: −13 ± 7%, t = 0.78, p > 0.05) compared to those in matched controls (n = 17). Further, CDK5 showed a strong and positive correlation with activator p35 (r = 0.72, p < 0.0001) but correlated poorly with p25 (r = 0.41, p = 0.05) in SZ PFC.

SZ subjects were then analysed according to the absence or presence of antipsychotic drugs in blood/urine samples. In SZ(−) subjects, p35, but not CDK5 or p25, was decreased (−52%) in PFC (Fig. 1a). In SZ(+) subjects under antipsychotic medication at the time of death, marked reductions of CDK5 (−47%), p35 (−76%) and p25 (−36%) were quantified in PFC (Fig. 1a). The down-regulation of p35 was greater in SZ(+) than in SZ(−), but the difference (24%) did not reach statistical significance (p = 0.07). The observed regulations of CDK5, p35 and p25 in SZ(−) and SZ(+) were reinforced when the corresponding pairs of matched subjects (control vs. SZ; matching variables: PMI; age; gender) were confronted (Fig. 1b). This analysis confirmed the reduction of p35 (−59%) in SZ(−) subjects, as well as the decreases of CDK5 (−44%), p35 (−77%) and p25 (−32%) in SZ(+) subjects (Fig. 1b).

CDK5 and activators p35/p25 in PFC/BA9 of MD subjects

The contents of CDK5, p35 and p25 in PFC of MD subjects, and subgroups of antidepressant-free and antidepressant-treated subjects, did not differ from those quantified in matched controls (Fig. 1c). Further analyses (paired t test) of the corresponding pairs of matched subjects (control vs. MD; matching variables: PMI; age; gender) did not detect alterations of CDK5, p35 or p25 in the groups and subgroups of MD subjects (data not shown).

Interrelationships between CDK5/p35/p25 and munc18-1a and syntaxin-1A in PFC/BA9 of SZ subjects

Given that CDK5 acts as a strong suppressor of neurotransmitter release and that this kinase regulates munc18-1α×syntaxin-1A interaction, two key proteins of the exocytotic machinery (see Introduction), one might expect significant interrelationships between the abnormalities of CDK5, p35 and/or p25 (current study) and those reported for munc18-1α and/or syntaxin-1A in the same SZ brain samples (Gil-Pisa et al. 2012). In PFC of SZ subjects (n = 24), there were positive and significant correlations between the content CDK5, p35 or p25 and that of munc18-1a (Fig. 2a). Positive relationships were also observed in the subgroups of SZ(−) and SZ(+) subjects, although the correlations between CDK5 and munc18-1a were the most relevant (data not shown). In contrast, CDK5 and activators p35/p25 did not correlate with syntaxin-1A content in the same SZ PFC samples (Fig. 2b), including the subgroups of SZ(−) and SZ(+) subjects. It is noteworthy that munc18-1α content did not correlate with that of CDK5 (r = 0.315, p = 0.219), p35 (r = 0.088, p = 0.736) or p25 (r = 0.042, p = 0.179) in PFC of control subjects (n = 17).

Discussion

In the context of the presynaptic hypothesis of SZ (e.g. see Fung et al. 2011), several dysfunctions of the exocytotic machinery have been reported in the post-mortem human brain (Johnson et al. 2008). Recently, abnormal munc18-1α×syntaxin-1A interaction and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex have been revealed in PFC of SZ subjects (Gil-Pisa et al. 2012). Most important was the finding that munc18-1α, synaptobrevin and SNARE contents in SZ PFC were down-regulated by antipsychotic medication, which may result in a destabilization/impairment of neurosecretion (Gil-Pisa et al. 2012).

It is well documented that CDK5 activation regulates munc18×syntaxin-1A interaction (Fletcher et al. 1999; Shuang et al. 1998) and that this kinase behaves as a suppressor of neurotransmitter release (Chergui et al. 2004; Kim & Ryan, 2010). One important finding of the current study strongly suggests that antipsychotic...
medication is associated with down-regulation of CDK5 and activators p35/p25 in SZ PFC (which would favour neurosecretion), indicating a compensatory mechanism to counteract the opposite action of antipsychotics on the exocytotic machinery (e.g. reduced munc18-1a and SNARE complex, which would dampen neurosecretion). The good interrelationship (same SZ PFC samples) between CDK5, p35 or p25 and munc18-1a, but not syntaxin-1A, is in line with the reported effects of antipsychotic drugs on CDK5 and munc18-1a in SZ (current study; Gil-Pisa et al. 2012). Interestingly, the reported changes in SZ brains were not observed in MD brains.
regardless of antidepressant medication, which appears to indicate some specificity in SZ concerning the abnormal process of neurosecretion and the effects of antipsychotic drugs in the PFC (current study; Gil-Pisa et al. 2012). It is also noteworthy that the basal content of activator p35 was decreased in PFC of antipsychotic-free SZ subjects, which indicates reduced CDK5 activation and, consequently, a possible increase in neurosecretion as a specific feature of SZ brain. In this context, however, a limitation of the current study is the relatively small sample size of MD subjects ($n=13$) analysed compared to the number of SZ subjects ($n=24$).

On the other hand, violent suicide was the main cause of death in SZ ($n=22$; all but two subjects) and MD ($n=12$; all but one subject), regardless of psychotropic drug exposure. It has been suggested that suicidal behaviour and completed suicide may have a distinct neurobiology (Pandey & Dwivedi, 2010). However, the reported abnormalities of CDK5/p35/p25 in PFC were observed in SZ but not in MD, which indicates that these brain targets are not specific to suicide.

There have been few studies on the status of CDK5 and activators p35/p25 in SZ (current study; Engmann et al. 2011; Funk et al. 2012; Swatton et al. 2004) and none, to our knowledge, in MD post-mortem brains (except the current study reporting negative data). An early study suggested an increase of p25 content in SZ PFC, but only three cases were analysed without indication of received treatments (Swatton et al. 2004). A recent study has shown a reduction of p35 protein expression, with normal CDK5 and p25 content, in PFC and hippocampus of SZ subjects, but blood–drug levels or medications received by SZ subjects at the time of death were not given (Engmann et al. 2011). Another recent study has reported a small but significant increase of CDK5 content, regardless of antipsychotic medication, in SZ PFC (Funk et al. 2012). The discrepancies between the present and other post-mortem brain studies on the regulation of CDK5 and activators p35/p25 in SZ PFC may be related to many different variables (see Gil-Pisa et al. 2012), which include the effects of previous exposure to antipsychotic or other psychoactive drugs as one of the most important factors.

In conclusion, the study indicates that SZ, but not MD, appears to be associated with reduced basal content of activator p35 (the main cofactor for CDK5 stimulation) in PFC of antipsychotic-free subjects. Moreover, SZ subjects under antipsychotic medication at the time of death showed marked down-regulation of CDK5 and activators p35/p25 in PFC. Further research is needed to determine whether these findings are specific features of SZ or are
induced by antipsychotic drug treatment. Noteworthy, cortical p35 was not altered by chronic clozapine treatment in rats (Engmann et al. 2011), which further suggests that the observed reduction of basal p35 in the PFC is related to SZ. The results also show that CDK5 and munc18-1a correlated in SZ PFC, suggesting a functional interaction that may lead to dysregulated neurosecretion.

Supplementary material
For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145712000879

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