Olanzapine, but not haloperidol, enhances PSA-NCAM immunoreactivity in rat prefrontal cortex

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Abstract
Repeated antipsychotic treatment may produce adaptive changes ranging from cytoarchitectural rearrangements to synaptic modifications that might contribute to clinical improvement. We performed a prolonged treatment (2 wk) with the first-generation antipsychotic (FGA) haloperidol (1 mg/kg) and the second-generation antipsychotic (SGA) olanzapine (2 mg/kg twice daily) and analysed the expression of the polysialylated form of neural cell adhesion molecule (PSA-NCAM) in rat hippocampus and prefrontal cortex via immunohistochemistry. We found a regional- and drug-selective increase of PSA-NCAM expression in prefrontal cortex of olanzapine-treated rats with no effects in hippocampus; conversely, haloperidol did not produce a change in either brain region. Our findings reveal a possible role for PSA-NCAM in the mechanism of action of the SGA olanzapine adding complexity as well as specificity to the molecular changes set in motion by this drug.

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Introduction
Antipsychotic drugs represent the first choice for the treatment of schizophrenia. Although all antipsychotics are effective in the treatment of psychotic symptoms, second-generation antipsychotics (SGAs) appear to have a better impact on negative symptoms and cognitive deterioration with respect to first-generation antipsychotics (FGAs) (Tamminga, 2003). These classes of drugs clearly present a different receptor profile, with haloperidol mainly blocking dopamine D2 receptors whereas olanzapine has a multireceptor profile with the primary antagonistic action at dopamine D2 and 5-HT2A receptors.

Besides the well-documented neurotransmitter alterations, schizophrenia is characterized by morphological abnormalities in some brain regions and dysfunction of neuronal plasticity, which might be linked to developmental events (Weinberger and Lipska, 1995). Hence, the therapeutic efficacy of antipsychotics might be related not only to their receptor profiles, but rather to their ability of modulating neuroplastic processes in selected regions.

The polysialylated form of neural cell adhesion molecule (PSA-NCAM) is abundantly expressed at late embryonic stages whereas at adulthood its expression is confined to brain regions characterized by persistent neurogenesis and plasticity, such as hippocampus and prefrontal cortex (Bonfanti, 2006; Seki and Arai, 1991a, b). To this end, recent evidence indicates that, at adulthood, PSA-NCAM expression is modulated by several conditions, such as stress (Cordero et al., 2005; Nacher et al., 2004; Sandi et al., 2001) as well as learning and memory tasks (Sandi et al., 2004; Senkov et al., 2006).

Given the role of PSA-NCAM in neurodevelopment, synaptic plasticity and cognitive processes that
are defective in schizophrenia, and the reduction of its expression in schizophrenics (Barbeau et al., 1995), we decided to analyse PSA-NCAM expression after repeated treatment with two prototype antipsychotics, olanzapine and haloperidol.

Methods and materials

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 225–250 g, aged 7–8 wk, were housed for 2 wk and maintained under a 12 h light/dark cycle (lights on 07:00 hours) with food and water available ad libitum. Ten rats for each experimental group were subcutaneously injected for 14 d with vehicle, olanzapine (2 mg/kg twice daily) and haloperidol (1 mg/kg once daily) and sacrificed 24 h after the last drug injection. The length of treatment has been demonstrated to produce adaptive changes which depend on the repeated exposure to the drug and not on the last injection (Fumagalli et al., 2006, 2008). Dosing regimens were based on literature data, considering biochemical (dopamine D$_2$ occupancy) (Andersson et al., 2002; Bymaster et al., 1996; Richelson, 1996; Schotte et al., 1996) as well as behavioural parameters (efficacy in reverting PCP-induced deficit) (Abdul-Monim et al., 2006). All animal handling and experimental procedures were performed in accordance with the EC guidelines (EC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

Rats were transcardially perfused first with sodium phosphate buffer (PBS) and subsequently with 4% paraformaldehyde in PBS (PFA, pH 7.4). After perfusion, the brains were extracted, post-fixed overnight in 30% sucrose-4% PFA and then cryoprotected in OCT at $-80\,^\circ$C. Coronal sections (16 μm thick) of the brains were prepared at the cryostat, at levels equivalent to 3.2 mm rostral and 4.30 caudal with respect to Bregma of the Paxinos Atlas, corresponding to prefrontal cortex and dentate gyrus of hippocampus respectively (Watson, 1996).

PSA-NCAM immunohistochemistry

Five alternate sections from each brain were processed ‘free-floating’ for PSA-NCAM immunohistochemistry. After blocking in 1% normal goat serum (NGS; Dako, Glostrup, Denmark, sections were incubated overnight with a mouse anti-Men B monoclonal IgM antibody (Chemicon, Temecula, CA, USA), diluted 1:500 in 0.1 M PBS, containing 1% (w/v) bovine serum albumin (BSA; Sigma, Milan, Italy) and 1% (v/v) NGS and then exposed for 3 h to fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgM (Chemicon). Sections were finally stained with the nuclear stain Hoechst 33258 and coverslipped with Citifluor mounting medium (Thermo, Agar Scientific, Essex, UK).

Quantitative image analysis was performed with an AxioVision 4.5 image analysis system connected to a Zeiss fluorescent microscope with high-sensitive CCD video camera. The total numbers of PSA-NCAM immunoreactive cells on dentate gyrus and medial prefrontal cortex (mPFC) were counted at 20×. For each section, the number of cells was divided by the area of the region of interest, i.e. dentate gyrus or mPFC, and multiplied by the average of the area, and finally a mean was calculated for each brain. Data were expressed as mean ± S.E.M. for each experimental group and statistical analysis was performed with one-way ANOVA.

Results

In accordance with published results (Seki and Arai, 1991b; Varea et al., 2005), we found that PSA-NCAM was abundantly expressed in the dentate gyrus, with a much lower expression in the mPFC (Figure 1). In the mPFC, long-term treatment with olanzapine, but not haloperidol, produced a significant increase of PSA-NCAM immunoreactivity in comparison to vehicle-treated rats, 24 h after the last injection (Figure 2a). Conversely, in the dentate gyrus of the hippocampus, olanzapine and haloperidol produced a tendency towards a decrease in the number of PSA-NCAM immunoreactive cells, which did not reach statistical significance (Figure 2b).

Discussion

Our results demonstrate that repeated administration of olanzapine, but not haloperidol, increases the
number of PSA-NCAM immunoreactive cells in the mPFC, without significant changes in the hippocampal dentate gyrus, suggesting a region-specific effect of the SGA.

Several lines of evidence support the notion that antipsychotic drugs can determine neuronal remodelling in brain regions involved in schizophrenia symptomatology (Harrison, 1999), although the mechanisms have yet to be clarified. Since PSA-NCAM is a major player in structural plastic processes such as neurite elongation, cell migration and synaptogenesis as well as synaptic remodelling (Muller et al., 2008; Rutishauser and Landmesser, 1996; Seki and Rutishauser, 1998), which are compromised in schizophrenia (Frost et al., 2004), we can hypothesize a beneficial effect of olanzapine on neuromodulatory deficits in mPFC through changes in synaptic structure and connectivity, perhaps mediated by an increase of PSA-NCAM. Although we did not investigate the cellular phenotype of PSA-NCAM-expressing cells, it has recently been demonstrated that PSA-NCAM immuno-reactive cells in the mPFC are probably GABAergic interneurons (Varea et al., 2005). Since the GABAergic system is known to be altered in schizophrenia (Lewis et al., 2005), these considerations suggest a potential of olanzapine in modulating the cortical GABAergic system. In support of a specific effect on GABAergic inhibition, olanzapine is able to up-regulate the expression of reelin (Fatemi et al., 2006), a protein specifically expressed by cortical GABAergic interneurons whose levels are also reduced in schizophrenia patients (Impagnatiello et al., 1998).

Since PSA-NCAM plays a role in higher cognitive functions mediated by mPFC, such as reversal learning and working memory (Markram et al., 2007), the possibility exists that the up-regulation of cortical PSA-NCAM might contribute to the amelioration of learning processes elicited by olanzapine in animal models of schizophrenia (Abdul-Monim et al., 2006). Further studies, using different drugs and additional doses for each drug, will be required in order to ascribe this effect to SGA, compared to FGAs.

Moreover, the evidence that PSA-NCAM expression is reduced in the hilus region of the hippocampus of schizophrenic brains, as revealed by post-mortem studies (Barbeau et al., 1995), together with the observation that chronic stress, a precipitating event for schizophrenia, can affect polysialylation of NCAM in limbic regions (Cordero et al., 2005; Sandi, 2004), point to this adhesion molecule as a potential target for antipsychotic drug treatment. Interestingly, in line with our results, linkage studies have pointed to NCAM as a candidate gene of the disease (Lewis et al., 2003; Sullivan et al., 2007).

In conclusion, our results reveal a previously unappreciated regulation of PSA-NCAM in response to prolonged antipsychotic treatment, which could augment neuronal plasticity that is defective in the schizophrenic brain. The potential therapeutic relevance of our findings is strengthened by the similar effect obtained with chronic antidepressant administration (Sairanen et al., 2007; Varea et al., 2007), thus pointing to enhanced PSA-NCAM in the mPFC as a common effect of long-term treatment with psychotropic drugs.
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Statement of Interest

None.

References


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