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Repeated risperidone treatment increases the expression of NCAM and PSA-NCAM protein in the rat medial prefrontal cortex

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Abstract

The present study investigates whether the anti-schizophrenic drug risperidone may evoke changes in the expression of NCAM/PSA-NCAM proteins, an indispensable element in the remodeling of synaptic arrangements, in the medial prefrontal cortex (mPFC). Rats were treated with risperidone (0.2 mg/kg, i.p.) either once or repeatedly (once a day, for 21 days). The expression of NCAM and PSA-NCAM proteins was analyzed via western blot and immunohistochemistry at intervals of 3 h and 3, 6, and 9 days after the single or the last risperidone dose. Repeated (but not acute) administration of risperidone was found to increase the expression of NCAM-180, NCAM-140 and PSA-NCAM proteins at 3 or 6 days after treatment. PSA-NCAM immunoreactivity was found in cell bodies, perisomatic-like sites, and in the neuropil of the mPFC. Neither single nor repeated risperidone administration changed the number of PSA-NCAM neurons in the mPFC. In contrast, the repeated risperidone treatment increased the number of PSA-NCAM perisomatic-like sites and the length density of PSA-NCAM positive neuropil at 3 days after the last injection. The data obtained indicate that risperidone, given repeatedly, may promote the remodeling of the structure of presumably GABA-ergic interneurons and that it may evoke the rearrangement of the synaptic contact in the mPFC.

1. Introduction

Dysfunction of the medial prefrontal cortex (mPFC) is a prominent feature of pathophysiology in schizophrenia. Impairments of cognitive function have been related to abnormalities in synaptic connectivity, efficacy of neurotransmission and metabolism in the mPFC (Lewis and Moghaddam, 2006; Lewis and Gonzalez-Burgos, 2008; Moghaddam and Homayoun, 2008). With respect to the anatomy of the mPFC, postmortem studies have shown an increase in cell packing density without a change in the total number of cortical neurons, but with changes in the principal components of synapses, such as a decreased amount of cortical neuropil (i.e., the condensation of axon terminals and distal dendrites (Rajkowska et al., 1998; Selemon and

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Goldman-Rakic, 1999)). In addition, the expression of components of excitatory synapses (i.e., the number of dendritic spines in specific regions of the mPFC) have been reduced (Costa et al., 2001; Halim et al., 2003). With respect to the specific neurotransmitter, abnormalities of neocortical GABA-ergic inhibitory neurons (forming characteristic perisomatic cartridges by the chandelier class of parvalbumin-containing neurons on pyramidal cell bodies and the axonal initial segment) have been noted in schizophrenia (Volk and Lewis, 2002; Lewis et al., 2005; Daskalakis et al., 2007). Downregulation of the presynaptic GABA-ergic function in schizophrenia has been reported as a decrease in the expression of glutamic acid decarboxylase-67 (GAD-67) (Akbarian et al., 1995; Volk et al., 2000) and presynaptic GABA transporter-1 (GAT-1) (Woo et al., 1998; Pierri et al., 1999; Volk et al., 2001), followed by a reduction in the number of parvalbumin-positive interneurons (Hashimoto et al., 2003). An increase in the expression of the α1 subunit of the postsynaptic GABAA receptors in the postsynaptic site of the cartridge has also been observed in the mPFC of schizophrenic brains (Impagnatiello et al., 1998). The pathological picture of the mPFC is not only limited to intrinsic cortical elements, but should also be extended to the aberration of neuroanatomy of efferent modulator systems. Although the etiology of schizophrenia leading to anatomical and functional changes is not yet clear, it is thought to be the consequence of a complex interplay between genetic susceptibility and environmental factors that alter the developmental/constitutive plasticity of neural circuits (Tsankova et al., 2007; Duman and Newton, 2007). The above hypothesis prompted an investigation to determine if genes/proteins controlling brain plasticity are engaged in the pathophysiology of schizophrenia or if pharmacological agents ameliorating positive and negative symptoms of schizophrenia are able to alter the expression of factors that govern the plastic rearrangement in central nervous systems (Deutsch et al., 2008).

Among several candidate genes/proteins inducing cortical hypoplasticity in schizophrenia, are the proteins involved in synaptogenesis (for example, the cell adhesion molecule (NCAM) (Sullivan et al., 2007) and the extracellular matrix protein, reelin (Impagnatiello et al., 1998)). NCAM is an important element of cell-to-cell and cell-to-extracellular matrix contact, involved not only in constitutive alterations of cortical anatomy (Maness and Schachner, 2007; Sullivan et al., 2007; Dalva et al., 2007), but also in neurocognitive dysfunction in schizophrenia (Sullivan et al., 2007; Atz et al., 2007). The NCAM gene is located in the middle of a genomic region relatively strongly implicated by the meta-analysis in linkage studies of the etiology of schizophrenia (Lewis et al., 2003). Moreover, NCAM proteins play an important role in neuronal process outgrowth, synapse formation, and signal transduction in the immature and the adult brain (Sytnyk et al., 2006; Maness and Schachner, 2007). The level of the soluble fraction of NCAM (NCAM-EC) derived from proteolytic cleavage (shedding) of extracellular NCAM elements, with the potential antagonistic activity on the intact extracellular membrane-attached NCAM molecule, is elevated in the prefrontal cortex, hippocampus and cerebro-spinal fluid (CSF) of schizophrenics (Honer et al., 1997; Vawter et al., 1998). Moreover, as in clinical studies, transgenic mice overexpressing NCAM-EC show increased locomotor activity (modeling psychomotor agitation) and neuroleptic dependent deficits in prepulse-induced inhibition of acoustic startle response (modeling deficits of sensorimotor gating), and they are oversensitive to the behavioral effects of stimulant drugs and NMDA receptor antagonists. Interestingly, these schizophrenic-like behaviors are accompanied by a reduction in immunoreactivity for GABA-ergic synapses forming perisomatic innervation of principal neurons in the mPFC and in the amygdala (Pillai-Nair et al., 2005). Thus, several lines of evidence indicate that NCAM proteins might be responsible for functional and anatomical abnormalities linked with the pathology of schizophrenia. Such abnormalities mediated by NCAM cannot be limited only to NCAM itself since it is post-translationally modified by the attachment of negatively charged sialic acid residues with a large hydrated volume (specifically polysialic acid (PSA)) to one of the protein's extracellular segments. Such sialylation attenuates the adhesive properties of neurons and enables the rearrangement of cell-to-cell and extracellular matrix-to-cell contacts (Rutishauser, 2008). Interestingly, a decrease in the PSA-NCAM protein level for the postmortem hippocampus of schizophrenics has also been reported (Barbeau et al., 1995). The anatomical studies showing that the PSA-NCAM protein is localized on the GABA-ergic interneurons in the human (Varea et al., 2007c) and rat prefrontal cortex (Varea et al., 2005) support the idea that NCAM or PSA-NCAM molecules might be connected with the GABA-ergic dysfunction observed in schizophrenia.

It is so far unknown whether or not neuroleptics may also affect the level of NCAM/PSA-NCAM proteins and subsequently evoked plastic changes in the mPFC. Recent evidence indicates that the atypical neuroleptic risperidone, but not haloperidol, a typical neuroleptic changed the gene expression of cell adhesion molecules such as integrin, neural adhesion molecule F3 in the rat cortex (Feher et al., 2005). Additionally, the available data indicate that PSA-NCAM expression in the mPFC is changed by chronic antidepressant administration via enhancement of serotonin transmission (Sairanen et al., 2007; Varea et al., 2007a,b). Thus, it is of interest to investigate whether treatment using risperidone, a neuroleptic acting as a D2/5HT2 receptor antagonist, which is commonly used in the treatment of schizophrenia (Stathis et al., 1996), is capable of altering the expression of NCAM and PSA-NCAM proteins in the mPFC. Therefore, the present study investigated whether or not acute and repeated risperidone administration influences the expression of NCAM and PSA-NCAM proteins in the rat mPFC using western blot and immnunohistochemical analyses to visualize the rate of PSA-NCAM/NCAM expression and the morphology of PSA-NCAM elements in the mPFC.

2. Experimental procedures

2.1. Animals and treatment

All of the experiments were carried out on male Wistar rats (Charles River). At the start of the experiments, they were 60 days old ("young adult rats") with body weights of approximately 200–250 g. Body weight changes were monitored during experiments and at the end of the treatment typical body weights were around 330–380 g with no differences between groups. The animals were housed on an artificial light/dark cycle (12/12 h lights on at 7 a.m.) with free access to a standard laboratory diet and tap water. The experimental protocols were approved by the Committee for Laboratory Animal Welfare and