Pharmacological Reports 68 (2016) 990–995

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Short communication

Extended neuroleptic administration modulates NMDA-R subunit immunoexpression in the rat neocortex and diencephalon

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ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 19 May 2016
Accepted 20 May 2016
Available online xxx

Keywords:
NMDA receptor
neuroleptics
glutamate
neocortex
diencephalon

ABSTRACT

Background: This study aimed to evaluate the effect of extended olanzapine, clozapine and haloperidol administration on NMDA-R subunit immunoexpression in the rat neocortex and diencephalon.
Methods: To explore NR1, NR2A and NR2B subunit protein expression, densytometric analysis of immunohistochemically stained brain slices was performed.
Results: Interestingly, all neuroleptics caused a downregulation of NMDA-R subunit expression in the thalamus but increased the level of NR1 in the hypothalamus. Olanzapine upregulated hypothalamic NR2A expression, while clozapine and haloperidol decreased hypothalamic levels. We observed no significant changes in NR2B immunoreactivity. None of the studied medications had significant influence on NMDA-R subunit expression in the neocortex.
Conclusions: Neuroleptic-induced reduction in the expression of thalamic NMDA-R subunits may play an important role in the regulation of glutamatergic transmission disorders in cortico–striato–thalamo–cortical loop in schizophrenia. A decrease in NMDA signaling in this region after long-term neuroleptic administration may also cautiously explain the incomplete effectiveness of these drugs in the therapy of schizophrenia-related cognitive disturbances.

Introduction

It has been established that during schizophrenia there are changes in NMDA receptor (NMDA-R) subunit composition in various regions of the brain [1–5]. However, current results are often inconsistent and our understanding of the influence of neuroleptics on NMDA-R expression remains incomplete. Nevertheless, on the basis of this data we can still try to create a hypothetical model of NMDA-R subunit expression changes in patients suffering from schizophrenia. In this model, NR1 subunit expression decreases in majority of brain structures of schizophrenia patients. Accordingly, these dysfunctions result from an NMDA-R dependent deficit in glutamatergic transmission in certain forebrain areas, such as the prefrontal cortex (PFC) and hippocampus [6–9]. The NMDA receptors indirectly regulate dopamine release in the brain cortex, suggesting that the dopaminergic hypofunction, typical in schizophrenia, is secondary to glutamatergic action [10]. Alternatively, dopaminergic receptors participate in the regulation of NMDA-R expression [11], and therefore, a final consensus on the sequence of signaling events underlying schizophrenia remains open. The ionotropic NMDA-R is a heterotetramer, composed of one mandatory subunit NR1, and three different subunits: NR2, NR3 and NR4 [12,13]. At present, eight splice variants of subunit NR1, and at least four classes of the subunit NR2: A, B, C and D have been identified. In patients suffering from schizophrenia, NR1 and NR2A subunit expression decreases in dorso-lateral PFC [14,15]. Transport of NR2B subunits is also disturbed, a lack of which can in turn impair NMDA-R function [16]. In frontal and occipital cortex there is an increase of NR2A subunit expression [17]. On the other hand, in the temporal cortex an increase of the number of NR2B subunits has been observed [18]. NR1 subunit expression is probably diminished in the thalamus [17] with simultaneous increase of the number of NR2B subunits in the dorso-medial area [19]. However, some

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http://dx.doi.org/10.1016/j.pharep.2016.05.009
1734-1140X/

studies have revealed no abnormalities in the thalamus [18]. In the hippocampus NR1 subunit expression decreases, while formation of NR2B subunits probably increases [20]. Moreover, the number of NMDA-Rs increases in putamen [21]. As the NR1 subunit is present in all NMDA-R, the results concerning putamen can be indirectly interpreted as an overall increase in the number of NR1 subunits. Similarly, expression of NR1 subunit in substantia nigra is probably decreased in schizophrenia [17]. Described changes of NMDA-R subunit composition can result from disease pathogenesis or from the influence of neuroleptics. Indeed, antipsychotics can change NMDA-R subunit gene expression and lead to modifications of receptor function [22]. We previously examined an effect of chronical treatment with haloperidol, clozapine and olanzapine on NMDA-R subunits expression in the rat hippocampus [23]. In the present article we aim to report and discuss the effect of these neuroleptics on NMDA receptor composition in the rat hypothalamus, thalamus and neocortex.It is especially worth mentioning that haloperidol is a classical neuroleptic with a widespectrum of unfavorable dyskinetic and cardiac side effects [24,25], while olanzapine and clozapine are new generation atypical medications which are more suitable in drug resistant schizophrenia [26–28]. In the context of this, comparing potential effects of these drugs on NMDA-R immunoexpression in various brain regions seems to be relatively novel and valid.

Materials and methods

Adult (5 month old, 210-240 grams weight) male Sprague-Dawley rats from Medical University of Silesia Experimental Centre were housed at 22 °C with regular 12/12 light-dark cycle andaccess to standard Murigran chow and water *ad libitum*.

Four groups of animals (n = 6) received either control vehicle, haloperidol (1 mg/kg/day), clozapine (20 mg/kg/day) orolanzapine (10 mg/kg/day) by intraperitoneal injection for 4 weeks. Three hours after the last drug administration, rats were anaesthetized with isoflurane andtheir brains removed and fixed in 4% paraformaldehyde PBS (pH 7.2-7.4). 7 μmthick sections were cut on amicrotome (Leica Microsystems, Germany) in the coronal plane (−2.60 to −2.00) from bregma, according to Paxinos& Watson's The Rat Brain in Stereotaxic Coordinates [29].

Sections were blocked with 5% goat serum and incubated with the following rabbit polyclonal primary antibodies against the NMDA receptor subunits; anti-NR1 (1:1500; AB17345), anti-NR2A (1:500; AB14596) and anti-NR2B (1:400; AB65875) were purchased from Abcam Company, Cambridge, UK. The Western blotting was made for each subunit separately, using the same antibody which was used at the IHC. The bands obtained could be a proof of the primary antibody specificity. To check the secondary antiserum we used negative control at each slide. It means that at one slide we had two sections of the same brain region. At all examined slides the negative control were blank.

Primary antibodies were followed by biotynylated goat anti-rabbit secondary antibody, and then visualized *via* avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector Labs), and 3,3′-diaminobenzidine. Sections were mounted on gelatin-coated glass slides, dehydrated and coverslipped.

The same level of cortical, thalamic and hypothalamic sections were chosen from each slide, and three standarized areas were studied (Fig. 1.). The relatively poorly investigated somatosensory cortex (barrel field S1BF) was examined. The hypothalamic frame contained ventro- and dorsomedial (VMH, DMH) as well as paraventricular nuclei (PVN) and lateral area (LH). In the thalamus we focused on the ventrolateral (VL), ventral anterior (VA), ventral posterolateral (VPL) nuclei involved in the crucial sensory processing. The immunoreactivity of NR1, NR2A and NR2B, containing cells in the randomly selected regions, were measured as the integrated optical density (IOD) and a mean ± SEM was calculated. The data were collected and analyzed using Nikon optic systems and software Image ProPlus (Media Cybernetics, USA). Statistical analyses were performed using Kruskall-Wallis and HSD test. Differences were considered statistically significant at $p < 0.05$.

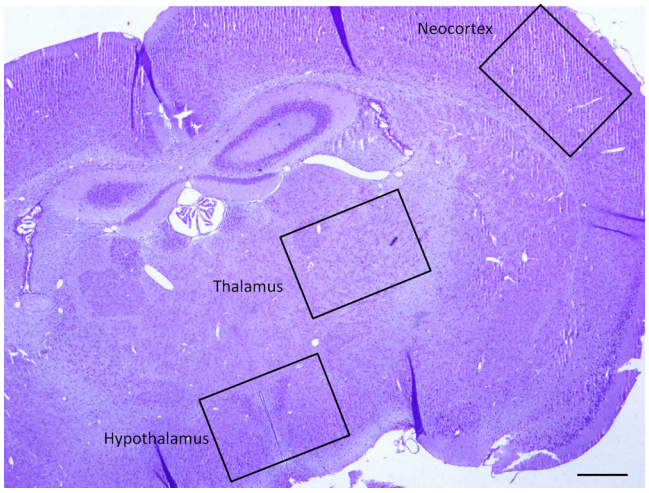

Fig. 1. Standarized frames used for the integrated optical density (IOD) measurement in the examined brain regions after immunohistochemical procedure. Nissl staining. Scale bar: 500 μm.

Results

Following neuroleptic treatment, changes in rat NMDA-R subunit expression were greatly dependent on the area of rat brain analyzed. In the thalamus each neuroleptic treatment caused a decrease in expression of all three NMDA subunits examined, most notably the general subunit—NR1. Interestingly olanzapine and clozapine caused equivalent reductions in each NMDA subunit (Figs. 2B and 3). In the hypothalamus all examined neuroleptics increased NR1 expression and also, in the case of olanzapine administration, we observed increased NR2A expression (Fig. 2A and B). However, treatment with clozapine and haloperidol caused reduced NR2A subunit expression in the hypothalamus. No significant changes in NR2B expression were observed in this region (Fig. 2A and B). In the cortex, drug treatment caused an increased trend in NR1 expression, but the result was not statistically significant. Moreover, none of the studied antipsychotics had significant influence on NR2A and NR2B expressions in the cortex.

Discussion

Collectively, these results suggest that the described neuroleptics inhibit the expression of NMDA receptors in the thalamus, which in turn can decrease the activity of the thalamic glutamatergic system. Alternatively, the NMDA-R subunit expression changes in the hypothalamus suggest an increase of glutamatergic circuit activity in this structure. In our analysis, we focused on the expression of the receptor subunits *via* protein quantification. Although, this may be a distinct limitation, we decided not to analyze expression at the mRNA level, given previous studies [30] and since changes at the mRNA level are rather difficult to interpret without specific information related to translation of receptor molecules [15]. For this reason, in the present experiment, we have emphasized a necessity of immunohistochemical assessment of the expression of NMDA receptor