Effect of Neuroleptic Administration on Neuronal Changes and Nitric Oxide in a Rat Model of Schizophrenia

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Abstract

Schizophrenia is a debilitating disorder that affects a considerable number of people worldwide with positive, negative and cognitive domains. The etiology of this disorder is not yet fully established. Frequently used medications to treat symptoms of schizophrenia include haloperidol and clozapine. While haloperidol is a typical neuroleptic commonly used for acutely ill patients, clozapine is an atypical neuroleptic and its prescription is restricted to refractory patients. Also, cerebrolysin has recently been used to treat cognitive deficits in schizophrenics. The neonatal ventral hippocampal lesion (nVHL) has emerged as a key model of schizophrenia related behavior producing numerous behavioral deficits, neuronal hypotrophy, reducing the number of neurons in the basolateral amygdala (BLA) and increasing nitric oxide (NO) levels. Our group has shown that clozapine and cerebrolysin reshape neurons in the prefrontal cortex (PFC), (BLA) and striatum. Cerebrolysin treatment also increases the spine density and the number of cells in the (PFC) in the (nVHL) rat. Moreover, clozapine and haloperidol target dopamine and serotonin neurotransmitter systems respectively, and (NO) modulates both of these systems. Thus the (nVHL) is a key model to understand schizophrenia and (NO) seems to be an ultimate effector.

INTRODUCTION

Schizophrenia is one of the diseases described in ancient medical writings. In every stage of the human history, attempts were made to explain this complex disorder with the available scientific elements. A new stage in understanding of schizophrenia occurred with the development of neuroleptics and antipsychotics in the 1950s. The first generation of neuroleptics include haloperidol and clozapine. While haloperidol is a typical neuroleptic commonly used for acutely ill patients, clozapine is an atypical neuroleptic and its prescription is restricted to refractory patients. Also, cerebrolysin has recently been used to treat cognitive deficits in schizophrenics. The neonatal ventral hippocampal lesion (nVHL) has emerged as a key model of schizophrenia related behavior producing numerous behavioral deficits, neuronal hypotrophy, reducing the number of neurons in the basolateral amygdala (BLA) and increasing nitric oxide (NO) levels. Our group has shown that clozapine and cerebrolysin reshape neurons in the prefrontal cortex (PFC), (BLA) and striatum. Cerebrolysin treatment also increases the spine density and the number of cells in the (PFC) in the (nVHL) rat. Moreover, clozapine and haloperidol target dopamine and serotonin neurotransmitter systems respectively, and (NO) modulates both of these systems. Thus the (nVHL) is a key model to understand schizophrenia and (NO) seems to be an ultimate effector.
in acutely-ill patients and in contrast clozapine prescription is restricted to refractory patients [7]. Recently, cerebrolysin is a preparation of peptides derived from porcine brain with effects on neuronal survival, adult neurogenesis and neuroplasticity [8,9]. Moreover, cerebrolysin mimics the action of endogenous neurotrophic factors [8], has shown cognitive improvement in schizophrenic patients [10]. The present manuscript review recent literature of the behavioral and neuronal effects of clozapine, haloperidol and cerebrolysin in the neonatal lesion of the ventral hippocampus (nLVH) model of schizophrenia-related behavior.

Hippocampus-prefrontal cortex pathway

Communication between the hippocampus and prefrontal cortex (PFC) has been known for several decades. Optimal connectivity is critical in the development of various behaviors, such as spatial memory. To better understand the function of a given structure, it is essential to describe how these structures are connected to different brain regions. At the end of the first month of gestation in humans, the neural tube is closed embryonic day 25. The six layers of the (PFC) and three layers of the hippocampus are fully established at gestational week (GW) 22 [11] as well as the connection between thalamus and (PFC) starts at the end of the five months of gestation and finishes 4 weeks later (GW)22-27 [11,12]. Interestingly, the connection between hippocampus, (PFC) and amygdala is established at the end of the seventh month of gestation [13,14]. Accordingly, there is an order of communication among various brain regions. When this order is altered, wrong communications may develop. Finally, between the eighth and ninth month of gestation, the communication between these structures receives and sends input to other regions as well. For example, both (PFC) and hippocampus send glutamatergic projections to nucleus accumbens NAcc [3]. The main population of neurons in the (PFC) and hippocampus is glutamatergic pyramidal neurons (Figure 2). Hippocampus may regulate the activity of (GABA)ergic neurons of the NAcc and medium spine neurons, directly and indirectly via (PFC) [3]. The medium spine neurons of the NAcc send (GABA)ergic projections to the ventral pallidum (Figure 2). The main neurons of the ventral pallidum send (GABA)ergic projections to the dorso-medial (DM) nucleus of the thalamus. Finally, the (DM) nucleus of the thalamus sends glutamatergic projections back to the (PFC) (Figure 3).

Neonatal ventral hippocampus lesion in rats

Pharmacological models of schizophrenia were predominant until the advent of the (nLVH) model. Developmental aspects of disrupting the prefrontal-hippocampus connectivity were addressed in this lesion [1,3,14-17]. Key research in schizophrenia in this model has been based on three aspects: multi-faceted behavioral effects, alterations of circuits and neurotransmitters and the periadolescent onset of abnormal neuronal and behavioral consequences, all of these symptoms are similar to what is seen in the schizophrenic patients [14]. All together numerous authors have suggested (nLVH) as a heuristic neurodevelopment model of schizophrenia. Indeed, (nLVH) rats present a delayed, post pubertal onset of behavioral changes such as locomotor hyper-responsiveness to novel environment.
Figure 2 This diagram illustrates the connections among the prefrontal cortex, the hippocampus and the basolateral amygdala, as well as the nucleus accumbens and dorso-medial (DM) thalamus. Blue lines indicate (GABA)ergic projections and red lines show glutamatergic projections.

Figure 3 This figure displays alterations in dendritic arborization and spine density in the pyramidal neurons of the prefrontal cortex (PFC), nucleus accumbens (NAcc) and basolateral amygdala (BLA) in adult rats with neonatal ventral hippocampus lesion (nVHL), an animal model of schizophrenia. Also, the schematic diagrams show how clozapine, an atypical neuroleptic leads to changes in the number of dendritic spines and dendritic arborization in this animal model of schizophrenia.

and stress, deficits in social interaction, sensor motor gating and learning and memory [3]. These behavioral changes after puberty are associated with decreased (DA) D3 receptors in the NAcc [16], increased nitric oxide (NO) levels in the (PFC) [18,19] and dendritic arbor atrophy and reduced spinogenesis in the (PFC) [17,18,20,21]. It is important to note that the behavioral and neurochemical effects of these lesions are different in several aspects if the lesions were performed in adult animals [22,23]. Therefore, the effects of this lesion may not be explained only in terms of the loss of ventral hippocampal neurons as the developing brain is an important additional factor.

Neuronal changes in the neonatal ventral hippocampus lesion before and after Neuroleptic administration

The shape of dendritic arbor of a neuron determines the number and distribution of receptive synaptic contacts [24]. Moreover, dendritic spines are the main site of input and therefore alterations in spine density results in either gain or loss of connectivity [25]. Modifications in dendritic arbor and spine
density disrupt neuronal stability. Neuronal rearrangement and alterations in spine density are observed in postmortem brains of the patients with schizophrenia [26] and animal models of schizophrenia-related behavior [3]. The (nLVH) induced hypertrophy and decreased dendritic spine density in the (PFC), basolateral amygdala (BLA) and NAcc [18,20,21,27]. In addition, the number of neurons is decreased in the (BLA) and (PFC) in (nLVH) rats [27]. Treatment with clozapine and cerebrolysin results in specific neuronal modifications in the (nLVH) rat. Clozapine increased the arborization in NAcc, (BLA) and (PFC) neurons with no modification in spine density [18] (Figure 3). Cerebrolysin increased the neuronal arborization without modifying the spine density in the (BLA). Moreover, cerebrolysin increased the number of cells in the (BLA) [27]. In the (nLVH) rat, cerebrolysin normalized the dendritic arborization and spine density in the (PFC) and arborization in NAcc neurons [21,27]. Finally, cerebrolysin treatment increased the number of cells in the (BLA) and (PFC) in the (nLVH) rat [21,27]. But the possible effects of haloperidol in dendritic arborization and spine density are yet to be described.

**Nitric oxide levels in the neonatal ventral hippocampus lesion before and after neuroleptic administration**

(NO) is a soluble, short-lived and freely diffusible gas considered as a key inter- and intra-cellular messenger [28]. (NO) is involved in numerous physiological processes including synaptic and neuronal plasticity that may produce functional modifications in brain circuits [29]. Indeed, processes known to be involved in the pathogenesis of schizophrenia [3] and accumulated evidence show involvement of (NO) in schizophrenia [30-32]. For example, schizophrenic subjects present increased level of plasma (NO) [33] and polymorphism of the neural nitric oxide synthase increases the genetic risk of schizophrenia [34]. Moreover, postnatal blockade of (NO) resulted in amphetamine- and novel-induced hyper locomotion and neuronal hypotrophy in the (PFC) and hippocampus [35,36]. In line with preclinical and clinical data, the (nLVH) rat presents increased levels of (NO) in the (PFC), occipital cortex (OC) and striatum [18,19,37]. Moreover, adult rats with (nLVH) also showed an increased (NO) S immunostaining in the (PFC) [38]. Clozapine administration decreased (NO) levels in the striatum with reduced locomotion in the (nLVH) rat [18]. This pharmaceutical also increased (NO) levels in the (PFC) and OC in sham animals with any behavioral modification [18]. Haloperidol reduced (NO) levels in the (PFC) and striatum as well as normalized locomotion in the (nLVH) rat [19]. But the effect of cerebrolysin on (NO) levels in the (nLVH) rat still needs to be investigated. However, recent reports show that the cerebrolysin treatment was able to reduce elevated hippocampal (NO) levels in an animal model of streptozotocin-induced diabetes mellitus [39]. In addition, several reports have demonstrated that cerebrolysin increases neurotrophins such as nerve growth factor (NGF) and brain-derived growth factor (BDNF) [40-42]. Both of these neurotrophins are implicated in synaptic plasticity [43].

Pioneer work from our group suggests that haloperidol and clozapine reduce behavioral deficits and neuronal hypotrophy in diverse key brain regions by modifying (NO) levels and display different receptor targets. However, it is well established that (NO) interacts with glutamate, serotonin and (DA) neurotransmitters [32]. In particular (NO) is a second messenger of N-methyl-Daspartate (NMDA) receptors, a subtype of glutamate receptors. Our working hypothesis suggests that upon activation of (NMDA) receptors, haloperidol and clozapine interact with dopaminergic and serotoninergic pathways [44]. Therefore both haloperidol and clozapine have been able to achieve similar behavioral and neuronal effects.

**CONCLUSION**

Schizophrenia is a devastating disorder with numerous symptoms negative, positive, and cognitive domains. Clinical and preclinical results suggest that this disorder present neuronal remodeling in several brain regions [3]. In particular, the (nLVH) model produced neuronal reshaping in the (PFC), (BLA) and NAcc [18,21,27]. The long term administration of clozapine as well as cerebrolysin reversed the behavioral deficits and normalized the dendritic arborization in (nLVH) rats. Moreover haloperidol and clozapine reduced the abnormal high levels of (NO) observed in (nLVH) rats. Furthermore, cerebrolysin also increased the number of cells in the (PFC) and (BLA). The beneficial effects of an increase in the number of cells may be based on antioxidant properties of cerebrolysin. Further studies are warranted to test the effects of other antipsychotics on (NO) levels and neuronal reshaping in the brain.

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**REFERENCES**


