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IMPACT OF POSTNATAL BLOCKADE OF *N*-METHYL-D-ASPARTATE RECEPTORS ON RAT BEHAVIOR: A SEARCH FOR A NEW DEVELOPMENTAL MODEL OF SCHIZOPHRENIA

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Abstract—The malfunction of glutamatergic neurotransmission in the neonatal or postnatal periods may be a risk factor for the appearance of neuroanatomical, neurochemical or functional changes that are characteristic of schizophrenia. Thus, the present study was undertaken to investigate whether blockade of *N*-methyl-D-aspartate (NMDA) receptors in the postnatal period influences rat behavior in tests characterizing schizophrenia-like deficits such as psychomotor agitation, impairments of sensorimotor gating, working memory, and intensity of social interactions. (E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116), a competitive antagonist of NMDA receptors, was given postnatally (1.25 mg/kg on days 1, 3, 6, 9; 2.5 mg/kg on days 12, 15, 18; and finally 5 mg/kg on day 21, all injections s.c.), and rats were tested at 60 days old. We found that blockade of NMDA receptors in the postnatal period led to an enhancement of exploration, mimicking psychomotor agitation, impairments in sensorimotor gating as measured by a prepulse-evoked inhibition of acoustic startle response, and an impaired working memory, as measured by an increase in the latency to achieve accurate rate of response in the delayed alternation task. Decreases in non-aggressive social interactions and increases in aggressive interactions were also observed. In addition to cognitive deficits typical of schizophrenia, rats treated postnatally with NMDA receptor antagonists also showed higher level of fear exhibited in the elevated plus maze. Thus, the blockade of NMDA receptors in the postnatal period may model deficits that are characteristic of schizophrenia.

Key words: developmental model of schizophrenia, NMDA receptors, working memory, sensorimotor gating, social interaction, psychomotor agitation.

Schizophrenia is a disorder which still has poorly defined etiology and pathophysiology. With heritability around 80% and appearance in monozygotic twins reaching 40% (Cardno and Gottesman, 2000), nongenetic or epigenetic factors must be involved as potential risk factors. Several clinical and epidemiological observations suggest that schizophrenia is the behavioral outcome of an aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms and is caused by a combination of environmental and genetic factors (Tsuang, 2000).

Neurodevelopmental hypotheses can be divided into two concepts; one favoring an early (pre- or perinatal), 'static' brain lesion model (Rapoport et al., 2005) and one arguing for disturbances in brain maturation arising in late adolescence (Feinberg, 1982). Available clinical data on samples obtained postmortem appear to support the early neurodevelopmental model as they report abnormalities in neuronal migration and organization (Rioux et al., 2003). Other well-documented observations such as reduced somal size and dendritic tree size of prefrontal neurons (Selemon and Goldman-Rakic, 1999; Kalus et al., 2000; Broadbelt et al., 2002), which could have developed later in life, indicate that the spectrum of disorders characteristic for schizophrenia results not solely from early developmental (prenatal or obsteric) complications. In experimental animals several methods have been used to model the developmental origin of schizophrenic symptoms. Such etiological models attempt to reproduce in experimental conditions some of the factors that are suspected to cause schizophrenia, such as obstetrical complications (Boog, 2004), particularly anoxia during birth (Vaillancourt and Boksa, 2000), bacterial and viral infections (Borrell et al., 2002; Ozawa et al., 2006), malnutrition (Palmer et al., 2004) or early stressful experience (Lehmann et al., 2000). Another group of models focuses on neonatal damage of brain regions implicated as a site of neuropathology in schizophrenia, mainly the hippocampus (Lipska et al., 1995a,b, 2002), but also other structures such as the prefrontal cortex (Lipska et al., 1998), thalamus (Volk and Lewis, 2003) and amygdala (Diergaarde et al., 2004, 2005). There have also been attempts to evoke alterations in cortical cytoarchitecture resembling those described in the brains of schizophrenic patients (Braff and Swerdlow, 1997; Kasai et al., 2002) by *in utero* exposure to factors directly interfering with neurogenesis, for example X-ray irradiation (Li et al., 2005) or administration of mitotic toxins such as methylazoxymethanol acetate (MAM) (for recent study see (Featherstone et al., 2007)) or cytosine arabinoside (Elmer et al., 2004).

Another group of developmental models of schizophrenia is based on the assumption that schizophrenia is a result of a transient or sustained reduction of glutamatergic neurotransmission (Goff and Coyle, 2001). This is a result

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Abbreviations: ANOVA, analysis of variance; CGP 40116, (E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; MAM, methylazoxymethanol acetate; NMDA, *N*-methyl-D-aspartate; post-CGP animals, rats treated postnatally with CGP 40116; post-Veh animals, rats treated postnatally with vehicle; PP, prepulse; PPI, prepulse-induced inhibition of acoustic startle response.

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of observations that ketamine (Krystal et al., 1994) and phencyclidine (Javitt and Zukin, 1991) evoke both positive and negative symptoms of schizophrenia in humans and their behavioral correlates in animals (Martinez et al., 1999; Becker et al., 2003). Moreover, mice with reduced levels of the NR1 subunit of the NMDA receptor display deficits typical of schizophrenia (Mohn et al., 1999). Finally, NMDA receptors are involved in brain maturation (McDonald and Johnston, 1990; Contestabile, 2000). Thus the concept of NMDA receptor hypofunction along with engagement of glutamate in developmental processes has given rise to several variations of animal models of schizophrenia based on postnatal NMDA receptor blockade. Repeated neonatal administration of phencyclidine evokes some behavioral alterations in adult animals such as reduction of baseline prepulse inhibition (PPI) of the acoustic startle response and retardation of the acquisition of a spatial alternation task (Wang et al., 2001). A single dose of ketamine during late gestation produces disturbances in learning and memory (Mickley et al., 2004). Perinatal subchronic administration of MK-801 also evokes impairments in working memory in adulthood (Stefani and Moghaddam, 2005) and disturbances of psychomotor activity (Schiffelholz et al., 2004).

Our recent studies revealed that (E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116), a competitive antagonist of NMDA receptors, given in a postnatal period (similar regimen of administration as described in the present paper) alters the structure of pyramidal neurons in the medial prefrontal cortex of adult rats (Wedzony et al., 2005b). These data support the neuropil theory of schizophrenia (Selemon and Goldman-Rakic, 1999) as the blockade of NMDA receptors in the postnatal period models morphological changes in pyramidal neurons of the medial prefrontal cortex, which are observed in some cases of schizophrenia (Kalus et al., 2000; Broadbelt et al., 2002). Moreover, it has been found that CGP 40116 given in postnatal period decreases the density of tyrosine hydroxylase terminals in the medial prefrontal cortex of adult animals (Wedzony et al., 2005a) i.e. evokes changes in the architecture of tyrosine hydroxylase terminals resembling the changes observed in the brains of schizophrenics (Akil et al., 1999). Finally, the postnatal blockade of NMDA receptors evokes enhancement of locomotor activity stimulated by quinpirole and amphetamine, which suggests the development of functional supersensitivity of subcortical dopaminergic systems modeling the appearance of positive symptoms of schizophrenia (Wedzony et al., 2005a). Thus, it was of potential interest to investigate whether the anatomical and pharmacological effects of CGP 40116 treatment are followed by other deficits modeling the behavioral outcome of schizophrenia. In contrast to other models based on administration of non-competitive antagonists of NMDA receptors like ketamine (Becker et al., 2003), phencyclidine (Martinez et al., 1999) or MK-801 (Schiffelholz et al., 2004; Stefani and Moghaddam, 2005), CGP 40116 is devoid of psychotomimetic properties (Wedzony et al., 1994). CGP 40116 is not able to evoke deficits in sensorimotor gating (Wedzony et al., 1994), and does not stimulate locomotor activity (Zajaczkowski et al., 2003). Moreover, CGP 40116 is capable of attenuating the psychotomimetic effects of non-competitive antagonists of NMDA receptors such as MK-801-induced locomotor hyperactivity and MK-801-evoked deficits in sensorimotor gating (Zajaczkowski et al., 2003). Thus the pharmacological profile of CGP 40116 and its administration in the postnatal period may help answer the question whether the blockade of NMDA receptors alone, or the blockade of NMDA receptors and experiencing of psychosis shortly after delivery (developmental models based on administration of MK-801, phencyclidine or ketamine) are needed for the appearance of schizophrenic symptoms in adulthood.

In a set of behavioral studies we investigate exploration, efficacy of sensorimotor gating, working memory and social interaction in animals treated postnatally with CGP 40116. We also evaluate the animals' anxiety, which often accompanies the cognitive symptoms of schizophrenia.

EXPERIMENTAL PROCEDURES

Animals

Pregnant dams (Wistar, Institute of Pharmacology Polish Academy of Sciences, Cracow) were housed under standard experimental conditions (constant temperature of 22 °C±2) with an artificial 12-h light/dark cycle (the light on from 7 a.m. to 7 p.m.). All subjects used in the experiment were born in the experimental facilities of Institute of Pharmacology Polish Academy of Sciences (Cracow). Dams and pups were housed in standard cages with wooden bedding and had free access to laboratory chow and tap water.

Administration of NMDA receptor antagonist

A day of parturition was designated as a postnatal day 0. Rat pups of both sexes were injected s.c. with increasing doses of CGP 40116. The drug dose was 1.25 mg/kg on days 1, 3, 6 and 9, then 2.5 mg/kg on days 12, 15, 18 and a final dose of 5 mg/kg was administered on day 21 (post-CGP group). The regimen of CGP 40116 administration has been taken from our previous studies where we demonstrated that the above dosage of CGP 40116 evoked alterations in the cytoarchitecture of the cerebral cortex of adult rats in a fashion similar to that observed in schizophrenia (Wedzony et al., 2005a,b) and produced enhancement of the locomotor activity evoked by amphetamine and quinpirole (Wedzony et al., 2005a), i.e. evoked supersensitivity of subcortical dopaminergic systems.

The volume of drug solution was 0.01 ml per 1 g of body weight. Control pups received only vehicle (NaCl, 0.9%, post-Veh group). Although the above regimen of drug administration was not lethal to any animals, on day P-21 we noticed a significant decrease in the body weight of post-CGP animals in comparison with the respective controls i.e. post-Veh rats (respectively, 43.7±0.9 vs. 54.5±0.9; $F_{(1, 98)}=69.1$; $P<0.001$). On day 60, when the behavioral tests were performed, the body weight of post-CGP and post-Veh animals was not significantly different (respectively, 244.5±5.3 vs. 256.4±5.3; $F_{(1, 98)}=3.4$; $P<0.07$ n.s.).

On postnatal day 22, the rats were separated from mothers and females were killed. Male rats, separately post-Veh and post-CGP, were randomly assigned to groups of six animals per cage. Except for the experiments with the delayed alternation task, rats were kept with water and food available *ad libitum*. In the delayed alternation experiments water was available *ad libitum* but food was restricted to 15 g per day and was available directly after