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Subsequent exposure to the choline uptake enhancer MKC-231 antagonizes phencyclidine-induced behavioral deficits and reduction in septal cholinergic neurons in rats

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Abstract This study examined the effects of subsequent, subchronic, treatment with choline uptake enhancer MKC-231 on the behavioral and cellular deficits induced by repeated PCP exposure in rats. Prior subchronic PCP exposure resulted in increased locomotion following an acute PCP or cocaine challenge, but resulted in decreased locomotor activity in response to a carbachol-challenge. MKC-231 significantly antagonized the alterations in the locomotor responses to cocaine and carbachol, but not to PCP. In the novel object recognition test, repeated PCP exposure caused cognitive deficits in rats, and the PCP-induced cognitive deficits were antagonized by MKC-231. In contrast, no effects of PCP exposure were shown in the repeated passive avoidance test. Furthermore, repeated PCP exposure decreased a number of choline acetyltransferase (ChAT)-positive cells in the medial septum and increased dynorphin A expression in the ventral striatum. Moreover, MKC-231 significantly antagonized the changes in septal ChAT-positive cells, but not the changes in ventrostriatal dynorphin A expression. These results suggest that MKC-231 could be a therapeutic drug for the treatment of schizophrenia.

1. Introduction

Schizophrenia is a devastating illness, producing positive (e.g. hallucinations, paranoia), negative (e.g. emotional withdrawal, motor retardation), cognitive and depressive symptoms. To examine the pathophysiology of schizophrenia, several animal models of schizophrenia have been developed (for a review, see Kilts, 2001). Among them, subchronic administration of phencyclidine (PCP) is thought to be a valid model of schizophrenia (Javitt and Zukin, 1991; Jentsch and Roth, 1999; Krystal et al., 2003; Morris et al., 2005). PCP performs its action through the antagonism of NMDA typed glutamate receptors (Javitt and Zukin, 1991). Repeated administration of PCP produced functional impairments in the hippocampus, amygdala, prefrontal cortex, posterior cingulate, and nucleus accumbens (Javitt and

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Zukin, 1991; Jentsch and Roth, 1999; Krystal et al., 2003; Morris et al., 2005).

Recent schizophrenia hypothesis consists of two main abnormalities of hyperdopaminergic tone and hypoglutamatergic tone (Duncan et al., 1999; Jentsch and Roth, 1999). Thus, drugs that block dopaminergic signaling reverse psychoses and drugs that reduce glutamatergic signaling cause psychoses. Furthermore, impairments of acetylcholine system are suggested (Javitt and Zukin, 1991; Carlsson et al., 2000). In experimental studies, the presence of PCP in the medium inhibited K^+-stimulated acetylcholine release in the striatal slices (Leventer and Johnson, 1983), and the NMDA receptor antagonist MK-801 decreased acetylcholine overflow in the striatum (Zocchi and Pert, 1994). However, subchronic PCP treatment did not reduce acetylcholine release in the prefrontal cortex (Jentsch et al., 1998). It appears that PCP affects acetylcholine signaling in a brain-region specific manner.

Acetylcholine and choline are critically involved in cognitive functions (Sarter and Parikh, 2005). Attentional processes need high levels of acetylcholine (for a review, Dalley et al., 2004). Two main cholinergic fibers are coming from the septum and nucleus basalis, and cholinergic interneurons exist in the brain. The hippocampus and prefrontal cortex are innervated by the medial septum and nucleus basalis, respectively (Helm et al., 2002). Previous studies reported that cortical cholinergic dysfunction was detected in schizophrenia (for a review, Sarter et al., 2005). Post-mortem studies demonstrated reduced numbers of muscarinic receptors in the prefrontal cortex, caudate–putamen and hippocampus of subjects with schizophrenia (Crook et al., 1999, 2000, 2001). A recent study revealed a decrease in the M1 muscarinic receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia (Dean et al., 2002). In vivo single photon emission computed tomography (SPECT) study showed decreases in muscarinic receptors in the prefrontal cortex, temporal cortex, caudate–putamen, and thalamus in patients with schizophrenia (Raedler et al., 2003). Furthermore, post-mortem studies demonstrated that choline acetyltransferase (ChAT) activities were high in the hippocampus, caudate putamen, nucleus accumbens, and amygdala of schizophrenic patients (Domino et al., 1973; McGeer and McGeer, 1977; Holt et al., 2005). McGeer and McGeer (1977) speculated that the high levels of ChAT in schizophrenia could be a compensatory response to defective cholinergic receptors in the affected areas. Therefore, it is reasonable that new muscarinic agonists have been developed for the treatment of schizophrenia (Shannon et al., 1999; Stanhope et al., 2001).

Atypical antipsychotic drugs are expected to be partially effective in treating cognitive deficits associated with schizophrenia. A recent study demonstrated that atypical antipsychotic drugs increase acetylcholine release in the medial prefrontal cortex, but not in the nucleus accumbens or striatum (Ichikawa et al., 2002). In addition, clozapine and olanzapine increase acetylcholine efflux in the hippocampus (Shirazi-Southall et al., 2002). Increased cholinergic activity was effective in treatment of the cognitive dysfunctions of schizophrenia (for a review, Friedman et al., 1999). Therefore, it could be that agents that strengthen the acetylcholine system are capable of antipsychotic activity (for a review, Miyamoto et al., 2005).

MKC-231, 2-(2-oxo-pyrrolidin-1-yl)-N-(2,3-dimetyl-5,6,7,8-tetrahydrofuro [2,3-*b*] quinolin-4-yl) acetoamide, is know to enhance choline uptake and acetylcholine release (Bessho et al., 1994), and it improved working memory deficits (Murai et al., 1994). The aim of this study is to examine whether MKC-231 could be beneficial for the treatment of schizophrenia. In this study, we performed three behavioral pharmacology experiments, open field test, novel object recognition test and passive avoidance test to examine the psychomotor excitement and cognitive impairments using subchronic PCP-treated animals. PCP, a dopamine agonist cocaine and a M1/M3 muscarinic receptor agonist carbachol was used as a challenge test in the open field test. To study the condition of acetylcholine system, we immunohistochemically examined a number of ChAT-positive cells in the septum and diagonal band. Finally, we examined expression of dynorphin A in the nucleus accumbens and striatum because hyperactivity in response to a cocaine-challenge is thought to be due to the association with increased accumbal dopamine.

2. Experimental procedures

2.1. Animals and treatments

Animal-use procedures were in accordance with the Tottori University Guide for the Care and Use of Laboratory Animals and were approved by the Tottori University Animal Care and Use Committee. Male Sprague–Dawley rats were housed individually under 12 hour light/dark conditions with free access to food and water. PCP hydrochloride, cocaine hydrochloride and carbachol were dissolved in physiological saline for intraperitoneal (i.p.) injection. MKC-231 was suspended in 0.5% Tween 80 solution for i.p. injection. As for PCP model of schizophrenia, rats received PCP (5 mg/kg, i.p., twice daily) or saline for 7 days. Subsequently, rats received a putative antipsychotic drug MKC-231 (3 mg/kg, twice daily) or saline for 8 days. Examined dose of MKC-231 was determined due to previous studies (Murai et al., 1994; Bessho et al., 1996). Next day, rats were administered behavioral tests or killed for immunocytochemical studies.

MKC-231 was kindly donated by Mitsubishi Pharma Corporation (Osaka, Japan). PCP and carbachol were obtained commercially from Sigma (St. Louis, MO, USA). Cocaine was purchased from Shionogi and Co. Ltd. (Osaka, Japan).

2.2. Open field test

Locomotor activity was measured in the open field test in a square area (76.5×76.5×49 cm) using a standard procedure (Lacroix et al., 1998). The open field was divided into two areas, a peripheral area and a square center (40×40 cm). The test room was dimly illuminated (two 60 W lights, indirect). Rats were allowed to explore for 45 min. The computer software (Be Trace: Behavioral and Medical Sciences Research Consortium, Hyogo, Japan) calculated the velocity of movement, the distance traveled, and time spent in the center of the open field. These parameters seem to reflect locomotor activity and fear or anxiety, respectively. PCP (1 or 10 mg/kg), cocaine (1.5 or 15 mg/kg), and carbachol (0.1 mg/kg) were intraperitoneally injected as a challenge test.

2.3. Novel object recognition test

The novel object recognition test was performed according to standard procedures with the following modifications (Tang et al.,