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Neurobiology of Learning and Memory 85 (2006) 86–94
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

The effects of clozapine on delayed spatial alternation deficits in rats with hippocampal damage

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Received 1 June 2005; revised 28 July 2005; accepted 24 August 2005
Available online 6 October 2005

Abstract

Clozapine is an atypical antipsychotic drug that has been shown to improve spatial memory in some animal models; however its efficacy in reversing spatial memory impairment in rats with hippocampal lesions is unknown. To address this issue, we tested the effects of clozapine on delayed spatial alternation deficits in rats with hippocampal damage in three separate experiments. In each experiment, adult male rats received sham surgery or direct stereotaxic infusions of the excitotoxin, NMDA, into the hippocampus. In the first study, seven days after surgery, the sham control animals received daily saline injections while the lesioned animals were split into two groups that received daily saline or clozapine (2.0 mg/kg, sc) injections. During the fifth week of injections, all animals were tested in a food-motivated delayed spatial alternation task. Saline-treated rats with excitotoxic hippocampal damage displayed significant deficits in delayed spatial alternation. Daily clozapine injections completely reversed this deficit. In a second experiment, it was found that clozapine treatment limited to the testing days only did not improve alternation performance in lesioned rats. Finally, in a third experiment, chronic clozapine treatment did not improve alternation performance in lesioned rats that were pre-trained in the alternation task prior to surgery. These results suggest that chronic, but not acute, clozapine treatment enables rats with hippocampal damage to develop new spatial learning, but can not rescue old spatial learning established prior to damage. These results may have implications for the treatment of cognitive deficits caused by hippocampal dysfunction in disorders such as schizophrenia, Alzheimer's disease, and others.

Keywords: Antipsychotic; Cognitive enhancement; Memory; Pre-training; Schizophrenia; Rats

1. Introduction

The atypical antipsychotic drug, clozapine, has been shown to improve cognition in people with schizophrenia. Studies have reported that chronic clozapine treatment improves psychomotor function and speed, attention, and/or verbal delayed recall memory in such people (Bilder et al., 2002; Lee, Jayathilake, & Meltzer, 1999; Sumiyoshi et al., 2004). Despite these demonstrations of clozapine's cognitive-enhancing properties, it is still unclear which receptors and brain regions mediate these effects or if they are consistent across all people with schizophrenia (Sumiyoshi et al., 2004).

Many animal studies have assessed the effects of clozapine on memory, in particular, spatial working memory. Alone, clozapine typically impairs spatial memory (Addy & Levin, 2002; Addy, Pocivavsek, & Levin, 2005; Didriksen, 1995; Skarsfeldt, 1996). However, when spatial working memory deficits are induced by pharmacological blockade of NMDA-type glutamate receptors or elevated dopamine release, clozapine actually improves memory performance in rats and monkeys (Hauber, 1993; Murphy, Roth, & Arnsten, 1997). These results are noteworthy since NMDA receptor hypofunction and dopaminergic hyperfunction have been routinely hypothesized to play a role in the pathophysiology of schizophrenia (see

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doi:10.1016/j.nlm.2005.08.010

Moghaddam, 2004; Winterer & Weinberger, 2004 for reviews).

Another neurobiological mechanism often implicated in the neuropathology of schizophrenia, as well as in other cognitive disorders such as Alzheimer's disease (AD), is altered size or shape of the medial temporal lobe – in particular, the hippocampus (Csernansky & Bardgett, 1998; Csernansky et al., 2000; McCarley et al., 1999). Evidence from neuroimaging studies suggests that reduced hippocampal volume is correlated with poor memory performance in people with schizophrenia or AD (de Toledo-Morrell et al., 2000; Gur et al., 2000). In animals, a multitude of studies have shown that damage to the hippocampus or one of its major inputs, the fimbria-fornix, disrupts spatial working memory (e.g., Bussey, Duck, Muir, & Aggleton, 2000; Dudchenko, Wood, & Eichenbaum, 2000; Hock & Bunsey, 1998). A recent study by Addy et al. (2005) demonstrated that while acute administration of clozapine to normal animals impairs spatial working memory, the same dose of clozapine improves memory in rats with lesions to the fimbria-fornix. However, it remains unknown whether the spatial working memory impairment produced by direct damage to the hippocampus is also sensitive to remediation by clozapine treatment.

The purpose of the present study was to determine if clozapine could improve memory performance in rats with direct excitotoxic damage to the dorsal hippocampus. Memory performance was assessed in a T-maze delayed spatial alternation task since this task had been used previously in animal studies demonstrating the cognitive enhancing properties of clozapine (Hauber, 1993; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996) and the deleterious effects of hippocampal damage on memory (Hock & Bunsey, 1998; Bussey et al., 2000; Dudchenko et al., 2000). We chose to first test the effects of chronic clozapine administration on memory performance since the clinical effects of most antipsychotic drugs are not immediate and are typically observed only after repeated treatment (Agid, Kapur, Arenovich, & Zipursky, 2003; Kuhar & Joyce, 2001). However, the effect of acute clozapine on memory function in rats with hippocampal damage was assessed in a second experiment.

In a third experiment, we considered the effects of pre-training on the ability of clozapine to modulate memory in rats with hippocampal damage. Aura and Riekkinen (2000) found that the cholinergic agonist, tetrahydroacridine (THA), and the NMDA receptor modulator, D-cycloserine, improved spatial memory in aged rats if each drug was administered during training. However, if the animals were pre-trained on the task prior to drug treatment, neither drug improved performance. The mechanism behind this phenomenon is unclear, but the findings suggest that some cognitive enhancing drugs may have selective effects (task familiarity/novelty, consolidation, or retrieval?) on memory. Given the debilitating effect of pre-training on the memory improving ability of two well-known cognitive enhancers, we determined if pre-training altered clozapine's action on memory in rats with hippocampal damage.

2. Materials and methods

2.1. Animals and housing

Adult male Sprague–Dawley rats (250–300 g) were used in this experiment. Animals were group-housed three per cage with free access to food and water except where noted. Lighting was maintained on a 12-h light/dark schedule with lights on at 07:30. All procedures were performed between 13:00 and 19:00. All experimental procedures were performed according to the Current Guide for the Care and Use of Laboratory Animals (USPHS) under a protocol approved by the Northern Kentucky University Animal Studies Committee.

2.2. Bilateral NMDA lesions of the dorsal hippocampus

Stereotaxic lesion surgery was performed in order to infuse *N*-methyl-D-aspartate (NMDA, Sigma) at a concentration of 12.5 mg/ml into the hippocampus. NMDA was dissolved daily in sterile saline. Following administration of Nembutal (Abbott) (65 mg/kg, IP), each rat was placed in a Kopf stereotaxic frame. A 1–2 cm long incision was made along the midline of the scalp. At each infusion site, a small burr hole was made in the skull with a small hand-held Dremel™ drill. Vehicle or NMDA was injected bilaterally along the longitudinal axis of the hippocampal formation at the coordinates and volumes listed in Table 1. Solutions were infused with a 2 μl Hamilton syringe attached to a 30-gauge needle via PE-10 tubing at the rate of 0.2 μl/min. The infusion needle was left in place for one minute after each infusion. Given the small size of the burr holes, they were not closed after infusions. The incision was closed with 3–4 wound clips and swabbed with betadyne scrub after the last infusion. Following surgery, animals received subcutaneous injections of six cc of saline to maintain hydration and were placed under a warm lamp until they regained mobility. Recovery from surgery was unremarkable in all animals, and incisions healed without any adverse reactions or problems.

2.3. Clozapine treatment

In the first and third experiments, drugs were injected subcutaneously into all animals once a day beginning one

Table 1
Stereotaxic coordinates for surgery

Anterior–posterior	Medial–lateral	Vertical	Infusion amount (μl)
−2.0	±1.2	−3.7	.15
−3.0	±1.6	−3.7	.08
−3.0	±3.0	−3.6	.15
−3.8	±2.0	−3.7	.08
−3.8	±3.6	−3.6	.15
−4.6	±2.9	−3.7	.08
−4.6	±4.0	−3.9	.15
−5.5	±3.7	−3.9	.15
−5.5	+5.0	−5.0	.15

All solutions were infused at a rate of .20 μl/min.