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ORIGINAL INVESTIGATION

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Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the $GABA_A$ receptor antagonist picrotoxin

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Abstract *Rationale:* Functional imaging studies have revealed overactivity of the hippocampus in schizophrenic patients. Neuropathological data indicate that hyperactivity of excitatory hippocampal afferents and decreased hippocampal GABA transmission contribute to this overactivity. In rats, excitation of the ventral hippocampus, e.g. by NMDA, results in hyperactivity and disruption of sensorimotor gating measured as prepulse inhibition (PPI) of the acoustic startle response, behavioral effects related to psychotic symptoms in humans. *Objective:* The present study examined whether disinhibition of the ventral hippocampus by the $GABA_A$ antagonist picrotoxin would result in similar psychosis-related behavioral disturbances (hyperactivity, decreased PPI) as NMDA stimulation. *Methods and results:* Wistar rats received bilateral infusions of subconvulsive doses of picrotoxin (100 or 150 ng/0.5 µl per side) into the ventral hippocampus and were then immediately tested for open field locomotor activity or startle reactivity and PPI. Only the higher dose induced hyperactivity and decreased PPI. Both doses decreased acoustic startle reactivity to a similar extent. The decreased PPI appeared not to result from decreased startle reactivity, but was associated with a diminished potency of the prepulses to inhibit the startle reaction to the startle pulse, indicating a sensorimotor gating deficit. All effects were temporary, i.e. disappeared when the rats were tested 24 h after infusion. *Conclusions:* Decreased GABAergic inhibition in the ventral hippocampus of rats yielded psychosis-related behavioral effects, very similar to those induced by NMDA stimulation. Thus, a concurrence of decreased GABAergic inhibition and increased afferent excitation in the hippocampus of schizophrenic patients might contribute to psychotic symptoms.

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Introduction

Animal studies concerned with hippocampal functioning receive particular interest, as aberrant hippocampal functioning in humans has been implicated in neuropsychiatric disorders such as anxiety and schizophrenia (Gray et al. 1991; Gray 1995; Benes 2000; Grace 2000). Functional imaging studies indicate that hippocampal activity is increased in schizophrenic patients and hippocampal overactivity has been associated with the experience of positive symptoms (Friston et al. 1992; Liddle et al. 1992; Silbersweig et al. 1995; Kawasaki et al. 1996; Heckers et al. 1998; Dierks et al. 1999; Frith 1999; Shergill et al. 2000).

In rats, overactivity of the ventral hippocampus, induced, for example, by infusion of NMDA to stimulate the NMDA-type receptor of the excitatory transmitter glutamate, generates behavioral effects which might be related to some positive symptoms occurring in schizophrenia. Locomotor activity in the open field is increased following NMDA stimulation of the ventral hippocampus (Yang and Mogenson 1987; Wu and Brudzynski 1995; Brudzynski and Gibson 1997; Bardgett and Henry 1999; Legault and Wise 1999; Bast et al. 2001b). Aberrant locomotor activity in the rat might be homologous to some changes in human cognitive function observed in acute schizophrenia (Gray et al. 1999). Furthermore, prepulse inhibition (PPI) of the acoustic startle response is disrupted following NMDA stimulation of the ventral hippocampus (Wan et al. 1996; Klarner et al. 1998; Koch et al. 1999; Zhang et al. 1999; Bast et al. 2001b). PPI is the reduction of the startle response to an intense acoustic pulse by an immediately preceding weaker stimulus, or prepulse. It may reflect sensorimotor gating mechanisms induced by the prepulse and preventing its processing from being inter-

rupted (Graham 1975; Norris and Blumenthal 1996). Deficient sensorimotor gating, as reflected by disruption of PPI, has been found in several neuropsychiatric disorders, especially in schizophrenia (Braff et al. 1978; Grillon et al. 1992; Perry and Braff 1994; Karper et al. 1996; Braff et al. 1999; Perry et al. 1999; Kumari et al. 2000; Parwani et al. 2000; Weike et al. 2000). Although there is little doubt about the existence of a PPI deficit in schizophrenia, its contribution to schizophrenic symptoms is not yet clear. However, deficient PPI has been postulated to contribute to sensory overload and cognitive fragmentation, which in turn result in psychotic symptoms, and correlations between decreased PPI and the severity of pychotic symptoms, in particular positive symptoms, have been reported by some studies (Perry and Braff 1994; Karper et al. 1996; Braff et al. 1999; Perry et al. 1999; Weike et al. 2000). Disrupted PPI in rats is used to model sensorimotor gating deficits observed in schizophrenia (Swerdlow et al. 1994, 2000a).

Overactivity of a brain structure can come about by increased excitatory neurotransmission and by disinhibition, i.e. a decrease of inhibitory transmission. In the hippocampus, inhibition is mainly mediated by actions of GABA, which is released by hippocampal interneurons, at the $GABA_A$ receptor, a ligand-operated Cl^--channel (Buhl et al. 1994). Neuropathological studies indicate that increased excitatory transmission via hippocampal afferents in concert with a local decrease of GABAergic inhibition (possibly due to a loss of hippocampal GABA interneurons) might yield the hippocampal overactivity revealed by functional imaging studies in schizophrenic patients (for review see: Benes 2000). Moreover, dysfunctions of GABA transmission have been implicated in the processes leading to psychosis (Keverne 1999; Lacroix et al. 2000) and psychotic symptoms in schizophrenia have been found to be correlated with reduced GABAergic inhibition in the medial temporal region (Busatto et al. 1997). Thus, decreased inhibitory GABA transmission in the hippocampus of schizophrenic patients might contribute to psychotic symptoms in schizophrenia.

In the present study, we tested in Wistar rats if the disinhibition of the ventral hippocampus by local microinfusion of the $GABA_A$ receptor antagonist picrotoxin would yield effects on PPI and locomotor activity in the open field that are similar to those resulting from stimulation of the ventral hippocampus by NMDA (Zhang et al. 1999; Bast et al. 2001b). In a recent study, bilateral infusion of 5 or 10 ng picrotoxin/side into the ventral hippocampus of Sprague-Dawley rats did not significantly affect startle amplitude or PPI (Japha and Koch 1999). The authors reported difficulties in testing higher doses because of convulsions occurring at higher doses. In our own pilot studies, doses up to 50 ng picrotoxin/side did not reveal any considerable behavioral effect or convulsive properties. Therefore, we used doses of 100 or 150 ng picrotoxin/side in the present study.

Materials and methods

Animals

A total of 50 male adult Wistar rats (Zur:Wist[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g at the time of surgery, were used in this study. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on: 1900–0700 hours) in a temperature (21±1°C) and humidity (55±5%) controlled room and were allowed free access to food and water. All rats received bilateral implantation of infusion guide cannulae aiming at the ventral hippocampus. After surgery, they were individually caged. Beginning 3 days before surgery and throughout the studies, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal experimentation.

Implantation of guide cannulae for intracerebral infusion

Rats were anaesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml; Abbott Labs, North Chicago, Ill., USA) per kg body weight and their head was placed in a Kopf stereotaxic frame. After application of a local anaesthetic (lidocaine), the scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the ventral hippocampus. Three small stainless steel screws were screwed into the skull and two guide cannulae (9 mm, 26 gauge; stainless steel) were implanted bilaterally into the brain through boreholes in the skull. The tips of the guide cannulae were aiming above the ventral hippocampus (5.2 mm posterior and ±5 mm lateral to bregma, and 5 mm ventral to dura). Guide cannulae were fixed by dental cement for which the three screws served as anchors to the skull. Stainless steel stylets (34 gauge) extending 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for 5 days during which the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets.

Intracerebral microinfusion

The rats were manually restrained, the stylets were removed from the guide cannulae, and infusion cannulae (34 gauge), connected to 10-µl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i), were inserted into the guide cannulae. The tips of the infusion cannulae protruded into the ventral hippocampus 1.5 mm beyond the tips of the guide cannulae, thus aiming at a final dorsoventral coordinate of 6.5 mm below the dura. The rats were bilaterally infused with 100 or 150 ng picrotoxin in 0.5 µl vehicle or 0.5 µl vehicle only. The infusion speed was 0.5 µl/min. After infusion, the infusion cannulae were left in the brain for 60 s to allow for absorption of the infusion bolus by the brain tissue and then replaced by the stylets. Rats were then immediately subjected to behavioral testing.

Drug preparation

The picrotoxin solutions were prepared freshly on the day of experiment. Picrotoxin ($C_{30}H_{34}O_{13}$; Fluka, Switzerland) was dissolved in 0.9% saline as vehicle to obtain concentrations of 200 or 300 µg/ml for infusion of 100 or 150 ng (0.17 or 0.25 nmol)/0.5 µl. The final pH was 6–7.