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Research report

Involvement of posterior cingulate cortex in ketamine-induced psychosis relevant behaviors in rats

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ABSTRACT

The involvement of posterior cingulate cortex (PCC) on ketamine-induced psychosis relevant behaviors was investigated in rats. Bilateral infusion of muscimol, a $GABA_A$ receptor agonist, into the PCC significantly antagonized ketamine-induced deficit in prepulse inhibition of a startle reflex (PPI), deficit in gating of hippocampal auditory evoked potentials, and behavioral hyperlocomotion in a dose dependent manner. Local infusion of ketamine directly into the PCC also induced a PPI deficit. Systemic injection of ketamine (3 mg/kg, s.c.) induced an increase in power of electrographic activity in the gamma band (30–100 Hz) in both the PCC and the hippocampus; peak theta (4–10 Hz) power was not significantly altered, but peak theta frequency was increased by ketamine. In order to exclude volume conduction from the hippocampus to PCC, inactivation of the hippocampus was made by local infusion of muscimol into the hippocampus prior to ketamine administration. Muscimol in the hippocampus effectively blocked ketamine-induced increase of gamma power in the hippocampus but not in the PCC, suggesting independent generation of gamma waves in PCC and hippocampus. It is suggested that the PCC is part of the brain network mediating ketamine-induced psychosis related behaviors.

1. Introduction

The posterior cingulate cortex (PCC) is part of the frontal cortex, and it is implicated in schizophrenia. In schizophrenia as compared to healthy controls, the PCC showed a decrease in gray matter [1,2], metabolic activity [3], or functional connectivity with the prefrontal cortex or temporal lobe [4,5]. Frontal temporal dysconnectivity has been proposed as a critical deficit in schizophrenia [6], and disruption of the default network mode [7], of which the PCC and hippocampus are components, may affect PCC function in internally-directed cognitive processes [8].

Ketamine, an uncompetitive NMDA (*N*-methyl-D-aspartate) receptor antagonist, is known to induce schizophrenia like symptoms in healthy humans [9] and worsen the symptoms of schizophrenia [10]. NMDA antagonist, MK-801, binding was increased in schizophrenia as compared to healthy subjects [11], and decreased metabolism in the PCC during episodic memory retrieval [12] may account for the cognitive deficits induced by ketamine.

Ketamine or another NMDA receptor antagonist also induces abnormal behaviors in animals, which serves as a model of schizophrenia [13,14]. We have shown that low doses (3–6 mg/kg s.c.) of ketamine induced a deficit in prepulse inhibition (PPI) and locomotor hyperactivity, and these psychosis-relevant behaviors were accompanied by a robust increase in gamma-frequency (30–100 Hz) local field potentials (LFPs) in the hippocampus [14,15]. In addition, ketamine and similar drugs also induced a decrease in sensory gating, measured by paired-pulse auditory evoked potentials in the hippocampus [16–18]. Medial septal inactivation, or selective lesion of septal GABAergic neurons, normalized both the electrophysiological and behavioral changes induced by ketamine [15,17,18]. The different physiological and behavioral measures are relevant for psychosis, because schizophrenic patients showed deficits in PPI [19] and gating of auditory evoked potentials recorded on the scalp [20,21]. High-amplitude gamma electroencephalogram (EEG) has been observed to correlate with positive symptoms in schizophrenia [22,23], and abnormal gamma synchronization may disrupt perceptual and memory processes [24–26].

The PCC is a key structure of limbic system with connections from the hippocampus, mainly through the subiculum [27–29]. The PCC is rich in NMDA receptors [30,31] and 40–100 mg/kg i.p. ketamine is known to induce pathological vacuoles [32] or activate c-Fos in the PCC [33,34]. In normal rats, LFPs in the PCC and hippocampus manifested both theta (4–10 Hz) and gamma frequencies [35,36], and theta coherence between PPC and hippocampus was found to increase with

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memory encoding and retrieval [36].

In this study, our goal was to study the involvement of the PCC in several psychosis relevant behavioral and electrophysiological measures induced by ketamine administration in rats. The behavioral measures include PPI and behavioral hyperlocomotion, and the electrophysiological measures include LFP recordings in the PCC and hippocampus, and gating of hippocampal auditory evoked potentials [14,17,18]. We were also interested in whether direct infusion of ketamine in the PCC activates psychosis-relevant behaviors, and whether local inactivation of the PCC suppresses the behaviors induced by systemic ketamine (3 mg/kg s.c.). Whether gamma waves are induced in the PCC by low-dose ketamine is not known, despite evidence of neuronal activation and pathology in the PCC after high doses (40–100 mg/kg i.p.) of ketamine [32–34]. Whether the gamma waves in the PCC are dependent on the hippocampus is also of interest. We hypothesize specifically that: 1) direct infusion of ketamine into the PCC induces a PPI decrease, 2) systemic ketamine (3 mg/kg s.c.) administration increases gamma oscillations in the LFPs of PCC, 3) the ketamine-induced gamma waves in PCC is independent of hippocampal gamma waves, 4) bilateral inactivation of the PCC by muscimol, a $GABA_A$ receptor agonist, blocks the loss in PPI and gating of auditory evoked potentials, and the increase in locomotor activity induced by systemic ketamine.

2. Materials and methods

2.1. Animals and surgery

Male Long-Evans hooded rats (Charles River Canada, St. Constance, Quebec, Canada) were housed in pairs in Plexiglas cages and kept on a 12/12 h light/dark cycle at a temperature of 22 ± 1 °C, with ad libitum food and water. All experimental procedures were approved by the local Animal Use Committee.

Under sodium pentobarbital (60 mg/kg i.p.) anesthesia, the rats were implanted with bilateral pairs of recording electrodes (125 μm Teflon-insulated stainless steel wires), each pair consisting of an electrode in stratum radiatum and stratum oriens of hippocampal CA1 region, respectively. The electrodes were targeted at AP −3.5, L ± 2.7, V 2.3 and 3.3 from skull surface (units in mm) according to the stereotaxic atlas of Paxinos and Watson [37]. The depth of each electrode pair was finalized by monitoring the recorded potentials evoked by stimulation of a contralateral hippocampal electrode, such that the electrode pair straddled the pyramidal cell layer [38]. Additional electrodes were implanted into the PCC layer V bilaterally at AP −2.3, L + 0.5, V2.5 from skull surface. In two experiments, 23-gauge (0.5 mm diameter), 14-mm long guide cannulae were bilaterally implanted for subsequent infusion into layer V in PCC (AP − 2.3, L ± 0.5, V 1.5) in one experiment, or hippocampal CA1 (AP −3.5, L ± 2.7, V2.3) in another. The LFP-recording electrode was glued to the outer wall of the guide cannulae. Small screws were implanted in the skull over the cerebellum and frontal cortex, to serve as recording grounds.

2.2. Local field potentials (LFPs) recordings in the hippocampus and posterior cingulate cortex

LFP recording electrodes were targeted at hippocampal CA1 stratum radiatum and layer V in PCC, also known as retrosplenial granular cortex [28,29]. PCC layer V, a layer with medium to large pyramidal cells, showed different patterns of LFPs, including slow irregular, theta and gamma activities [35]. The layer of recording in PCC is likely not critical because open, dipole fields in PCC [35] can be recorded at different layers. Recordings were made during baseline (before injection) spontaneous behaviors, and at fixed times after administration of drugs or vehicle. Spontaneous behaviors were grossly classified as either walking or awake immobility. Walking was operationally defined as horizontal and vertical movements and large body turning. Awake immobility was defined as standing or sitting still without gross movements of the body and head. Low-amplitude theta and gamma activities were observed during awake immobility, and high-amplitude theta and gamma oscillations were found during walking. Since behavioral immobility was rare after ketamine injection, hippocampal LFPs were recorded at fixed times after ketamine injection, and the behavior during the 0.5–1 min of recording was classified as immobility or walking. Since the rat was typically moving after ketamine, the post-ketamine LFP activity was compared using the walking LFPs as the baseline.

After baseline recordings, ketamine (3 mg/kg, s.c.) or saline (0.2 mL/per rat s.c.) was injected. In another experiment, bilateral infusion of saline or muscimol (0.5 μg/0.5 μL/side) was made into the hippocampus, followed 15 min later by ketamine (3 mg/kg, s.c.). LFPs were recorded in freely behaving rats at fixed time periods after ketamine/saline s.c. injections, and the behavioral state (walking or immobility) was noted. The LFP was digitized at 200 Hz and analyzed in 5.12-s segments (1024 points). Each LFP segment was tapered at the ends, fast Fourier transform, and 5 adjacent frequency bins were smoothed by an elliptical function to yield a power spectrum [39]. The average autopower spectrum was calculated from 0 to 100 Hz. Average power of gamma at 30–58 Hz (called low gamma) and gamma at 65–100 Hz (called high gamma) was calculated by averaging the linear power at all digitized frequency within the specified range and then presented as logarithmic power. The peak theta rhythm was measured as a rise in power, in logarithmic units, from a minimum at 1–5 Hz to a peak at 5–10 Hz. The coherence spectra between the LFPs in the hippocampus and PCC were expressed as z-transform coherence [39].

2.3. Gating of hippocampal auditory evoked potentials (AEPs)

Hippocampal electrodes were connected to a flexible cable that was led through an opening of the semi-restraining chamber. The latter chamber, a cylinder with a semi-circular cross section, restrained the rat to face one direction, and sound was delivered at 24 cm above the rat's head. AEPs at a CA1 stratum radiatum electrode were acquired following auditory click pairs called conditioning pulse (1st, C-pulse) and test pulse (2nd, T-pulse) were separated by an interval of 520 ms. Each click was a white-noise burst of 20 ms duration at 75 dB intensity, and click pairs were given 15 s apart. Single sweeps of the AEP were stored on the computer and those sweeps with clear movement or electrical artifacts were rejected online, and additional sweeps could also be rejected offline. Twenty-five sweeps were averaged in the average AEP, from which the ratio of peak amplitude of the T-pulse response to the peak amplitude of the C-pulse response (T/C ratio) was evaluated. Each rat was tested during baseline before infusion and injection, and then the PCC was infused with saline (0.4 μL/side) or muscimol (0.4 μg/0.4 μL/side) followed 15 min later by ketamine (3 mg/kg s.c.) injection. Post-injection AEPs were acquired immediately after ketamine injection. The T/C ratio, or paired-pulse response ratio, was commonly used in AEP analysis in animals [16] or humans [20], and a lack of auditory gating is indicated by T/C ratio > 1.

2.4. Quantification of locomotor activity

Locomotor activity was recorded in rats different from those used for PPI/AEP measurements. Horizontal movements (locomotion) of a rat were measured by the number of interruptions of infrared beams in a Plexiglas chamber (69 cm x 69 cm x 49 cm). Four independent infrared sources, at 23 cm intervals, were located on a horizontal plane 5 cm above the floor, with photodiode detectors on the other side. Interrupts of the beams were counted and transferred to a microcomputer via an interface (Columbus Instruments). A rat was habituated for 30 min in the chamber before injection, and infrared interruption counts per minute were recorded for 5 min baseline recording. The rat was then bilaterally infused with muscimol at a dose of 0.4 or 1 μg/0.4 μL, or with vehicle (0.4 μL) into the PCC, followed 15 min later