Phencyclidine Inhibits the Activity of Thalamic Reticular Gamma-Aminobutyric Acidergic Neurons in Rat Brain

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Background: The neurobiological basis of action of noncompetitive *N*-methyl-D-aspartate acid receptor (NMDA-R) antagonists is poorly understood. Electrophysiological studies indicate that phencyclidine (PCP) markedly disrupts neuronal activity with an overall excitatory effect and reduces the power of low-frequency oscillations (LFO; <4 Hz) in thalamocortical networks. Because the reticular nucleus of the thalamus (RtN) provides tonic feed-forward inhibition to the rest of the thalamic nuclei, we examined the effect of PCP on RtN activity, under the working hypothesis that NMDA-R blockade in RtN would disinhibit thalamocortical networks.

Methods: Drug effects (PCP followed by clozapine) on the activity of RtN (single unit and local field potential recordings) and prefrontal cortex (PFC; electrocorticogram) in anesthetized rats were assessed.

Results: PCP (.25–.5 mg/kg, intravenous) reduced the discharge rate of 19 of 21 RtN neurons to 37% of baseline ($p < .000001$) and the power of LFO in RtN and PFC to ~20% of baseline ($p < .001$). PCP also reduced the coherence between PFC and RtN in the LFO range. A low clozapine dose (1 mg/kg intravenous) significantly countered the effect of PCP on LFO in PFC but not in RtN and further reduced the discharge rate of RtN neurons. However, clozapine administration partly antagonized the fall in coherence and phase-locking values produced by PCP.

Conclusions: PCP activates thalamocortical circuits in a bottom-up manner by reducing the activity of RtN neurons, which tonically inhibit thalamic relay neurons. However, clozapine reversal of PCP effects is not driven by restoring RtN activity and may involve a cortical action.

Key Words: Antipsychotic drugs, clozapine, NMDA receptor antagonists, psychotic symptoms, schizophrenia, thalamocortical networks

The noncompetitive *N*-methyl-D-aspartic acid receptor (NMDA-R) antagonists phencyclidine (PCP) and ketamine are extensively used in pharmacological models of schizophrenia due to their ability to evoke and aggravate schizophrenia symptoms in healthy individuals and schizophrenic patients, respectively (1,2). PCP, ketamine, and dizocilpine (MK-801) also evoke behavioral alterations in experimental animals, which are partly or totally antagonized by antipsychotic drugs (3–5). The brain networks and cellular elements involved in these actions are not fully understood, yet neuroimaging studies indicate that these agents increase the activity of prefrontal cortex (PFC) and cingulate areas (6–10). Work in experimental animals has enabled identification of the actions of NMDA-R antagonists in various brain areas, including the PFC, the thalamus, and the hippocampal formation (5,11).

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Previous studies showed that PCP markedly altered the activity of pyramidal neurons in the medial PFC (mPFC) (12) and thalamic relay neurons of the centromedial (CM) and mediodorsal (MD) thalamic nuclei (13) in anesthetized rats. PCP had a mixed action in PFC and CM/MD neuronal activity, yet with an overall excitatory effect, resulting from the higher percentage of neurons excited (45% in PFC, 57% in CM/MD) and the large increase in neuronal discharge (286% in PFC, 424% in CM/MD). Concurrently, PCP reduced low-frequency oscillations (LFO; <4 Hz) in mPFC and CM/MD and increased *c-fos* expression in thalamocortical areas (12,13). PCP effects were blocked by antipsychotic drugs (12–14), which supports the association of these alterations to psychotic symptoms.

The primary cellular/regional site of action for these PCP effects is unclear. Given the reciprocal connectivity of the PFC and CM/MD (15,16), PCP blockade of NMDA-R inputs onto PFC gamma-aminobutyric acidergic (GABAergic) interneurons, as observed for MK-801 (17), would disinhibit projection pyramidal neurons and increase corticothalamic inputs (top-down process). PCP might also block NMDA-R inputs onto GABAergic neurons in basal ganglia structures and/or the reticular nucleus of the thalamus (RtN), which provide tonic feed-forward inhibition to thalamic relay neurons (bottom-up process). We conducted the present study to examine whether PCP inhibits the activity of RtN neurons in vivo, which, according to the working hypothesis, would reduce GABA inputs onto CM/MD nuclei and increase thalamocortical activity, as observed previously (12,13).

Methods and Materials

Animals and Treatments

Male albino Wistar rats weighting 250–320 g (Iffa Credo, Lyon, France) were used in this study. Animal care, treatments, and

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recording procedures were essentially as described (12,13) (see also Methods in Supplement 1). Animal procedures followed European Union regulations (Official Journal of the European Communities L358/1, December 18, 1986). Rats were deeply anesthetized (chloral hydrate, 400 mg/kg intraperitoneal [i.p.]). A constant level of anesthesia was obtained by infusing 50–70 mg/kg/hour chloral hydrate i.p. The femoral vein was cannulated for intravenous (i.v.) drug administration. Animals were implanted with a 2-mm screw fixed in the skull skimming the cortical brain surface (dura mater was accurately removed) for electrocorticogram (ECoG) measurements in prefrontal cortex (PFC; anteroposterior [AP] + 3.2, L −.5 and dorsoventral [DV] brain surface). RtN neurons (Figure 1) were recorded with glass micropipettes filled with 2 mol/L sodium chloride containing 2% pontamine sky blue dye (Avocado Research Chemicals Ltd., Lancaster, United Kingdom). Descents were carried out in the ipsilateral RtN (AP −1.4 to −3.14, L −1.8 to −3.8, and DV −5 to −6.5 mm below brain surface) according to the method described by Paxinos and Watson (18).

Drugs

PCP hydrochloride (.25–.5 mg/kg i.v.) was from Sigma/RBI (Natick, Massachusetts), and clozapine (CLZ; 1 mg/kg i.v.) was from Tocris (Bristol, United Kingdom). Doses were chosen from previous experiments (12,13).

Electrophysiological Experiments

Electrophysiological procedures were essentially as described previously (13). For single-unit, local field potential (LFP) and ECoG recordings, the original signal was amplified and filtered between 30 Hz and 1 kHz, .1–100 Hz, and .1–200 Hz, respectively. Sampling rate was 25 kHz for single-unit recordings and 2.5 kHz for LFP and ECoG recordings. Data storage was made with Spike2 software (CED, Cambridge, United Kingdom). RtN neurons were identified by their electrophysiological characteristics (19–24). RtN neurons exhibited firing rates higher than thalamic relay cells and with great variability in burst characteristics (see Results). Likewise, histological verification of the recording site was carried out. Neurons outside the RtN were excluded from the study.

Histological Procedures

Tissue treatment for localization of pontamine has been described elsewhere (13) (Methods in Supplement 1). Briefly, tissue sections adjacent to the pontamine dot were thaw-mounted onto individual slides. The two best consecutive sections containing an intense pontamine dot were processed, one for neutral red staining and the other for in situ hybridization of parvalbumin (PV) to label RtN cells (25). Low-magnification images of the two sections were then obtained and merged with Photoshop software (Adobe, San Jose, California) to verify electrode localization (Figure 1).

PV-positive cells were identified by an oligodeoxyribonucleotide probe complementary to bases 205–249 (GenBank accession number X63070.1; Isogen Bioscience BV, De Meern, The Netherlands). Labelling of the probes and in situ hybridization procedures were carried out as described previously (26,27) (Methods in Supplement 1).

Data Analysis

Electrophysiological data analysis was performed offline (12,13). A detailed description is given in Supplement 1 Methods. Briefly, each treatment condition (basal, PCP, and CLZ/saline) was recorded for 5 min, of which the last 2-min periods were used for data analysis. Burst analysis of RtN neurons was made as previously described (24). Burst strength was analyzed using interspike intervals (ISI) and defined as a burst index: ISI <10 ms divided by ISI <200 ms.

For LFP and ECoG recordings, signals were downsampled 16 times, and raw data were imported to MATLAB development environment (MathWorks, Natick, Massachusetts) for off-line analysis, using built-in and self-developed routines. LFP and ECoG analyses were performed in the same time segments as spike analysis.

To compare common oscillatory patterns between signals, wavelet coherence was first used (28) as a measure of power and phase difference stationarity. In this sense, stationarity periods implies phase differences in which mean and variance do not change over time. On the other hand, phase-locking values were defined specifically (29) as a measure of the stationarity of the phase differences in a temporal window and therefore of the phase-dependent synchrony (Methods in Supplement 1). Due to the specificity of the latter measurement, this was calculated by considering only the dominant oscillation. Likewise, we analyzed the phase locking between the dominant 1-Hz oscillation (.5–2 Hz) in PFC and discharge of RtN neurons by using the first spike of any burst event.

Differences between each treatment condition were evaluated with paired Student *t* tests and one- or two-way analysis of variance (ANOVA), followed by the Newman-Keuls test as post hoc tests, as appropriate. Data are means ± SEM, and significance was set at a *p* value of <.05.

Results

Firing Patterns of RtN Neurons

The effects of PCP were examined in a total of 21 RtN neurons (one per rat), localized in different sectors of the RtN, with a mean DV coordinate of 5.87 ± .09 mm ($n = 21$) (Figure 1). The spontaneous discharge rate of recorded neurons was 16.4 ± 1.2 spikes/sec ($n = 21$), nearly 10-fold that of CM/MD neurons (1.7 ± .2 spikes/sec) (13). Unlike thalamic relay neurons, RtN neurons fired in a variety of combinations of tonic and burst firing patterns. Some neurons fired exclusively in tonic mode whereas others fired only in burst mode. The average burst index was .37 ± .04 ($n = 21$), within the range of reported values (24). Figure 1C–E shows representative examples of firing patterns of RtN neurons.

Effect of PCP on the Discharge of RtN Neurons

Overall, PCP administration (.25–.5 mg/kg i.v.) markedly reduced the discharge rate of RtN neurons from 16.4 ± 1.2 to 6.7 ± .9 spikes/sec ($p < .000001$, paired Student *t* test, $n = 21$). Nineteen neurons were inhibited by PCP from 16.9 ± 1.2 to 6.2 ± .9 spikes/sec (37 ± 5% of baseline; $p < .000001$), and two neurons did not respond (from 11.6 ± .7 to 11.8 ± .5 spikes/sec) despite the fact that PCP simultaneously reduced the power of LFO in PFC (see below). The effect of PCP persisted for at least 10 min (from 13.8 ± 1.5 to 4.4 ± 1.2 and 3.4 ± .9 spikes/sec under basal conditions, 5 and 10 minutes after PCP administration, respectively; one-way ANOVA: $F_{2,10} = 37.72$, $p < .001$; $n = 6$). Figure 2A shows a representative example of the effect of PCP on the discharge of an RtN neuron. PCP did not alter the firing pattern of inhibited neurons. Burst index were 0.36 ± .04 and 0.39 ± .08 under basal conditions and after PCP administration, respectively (nonsignificant, paired Student *t* test, $n = 19$).

Effect of PCP on LFO in RtN and PFC

In parallel with the effects on the discharge of RtN neurons, PCP significantly reduced the power of LFO (.2–4 Hz) in RtN and