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Dopamine dynamics during emotional cognitive processing: Implications of the specific actions of clozapine compared with haloperidol

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ABSTRACT

Clozapine has improved efficacy relative to typical antipsychotics in schizophrenia treatment, particularly regarding emotional symptoms. However, the mechanisms underlying its therapeutic benefits remain unclear. Using a methamphetamine-sensitised rat model, we measured changes in dopamine levels in the amygdalae in response to a fear-conditioned cue, serving as a biochemical marker of emotional cognitive processing disruption in psychosis, for analysing the biochemical mechanisms associated with the clinical benefits of clozapine. We also compared how clozapine and haloperidol affected basal dopamine levels and phasic dopamine release in response to the fear-conditioned cue. Extracellular dopamine was collected from the amygdalae of freely moving rats via microdialysis and was analysed by high-performance liquid chromatography. Clozapine or haloperidol was injected during microdialysis, followed by exposure to the fear-conditioned cue. We analysed the ratio of change in dopamine levels from baseline. Haloperidol treatment increased the baseline dopamine levels in both non-sensitised and sensitised rats. Conversely, clozapine only increased the basal dopamine levels in the non-sensitised rats, but not in the sensitised rats. Although both antipsychotics attenuated phasic dopamine release in both the non-sensitised and sensitised rats, the attenuation extent was greater for clozapine than for haloperidol under both dopaminergic conditions. Our findings indicate that stabilized dopamine release in the amygdalae is a common therapeutic mechanism of antipsychotic action during emotional processing. However, the specific dopaminergic state-dependent action of clozapine on both basal dopamine levels and stress-induced dopamine release may be the underlying mechanism for its superior clinical effect on emotional cognitive processing in patients with schizophrenia.

1. Introduction

The efficacy of clozapine is improved compared with typical antipsychotics in schizophrenia treatment (McEvoy et al., 2006) and is beneficial against suicidal behaviour, emotional deficits, negative symptoms and cognitive dysfunction (Krakowski et al., 2006). Because emotional symptoms represent a key individual factor inhibiting normal social functions in individuals with schizophrenia (Lysaker and Salyers, 2007), emotional processing is considered a useful therapeutic target. However, the mechanisms of the superior clinical effect of clozapine on emotional symptoms in patients with schizophrenia have not been completely elucidated.

Basolateral complexes of the amygdalae are among the most potent modulators of emotional memory in animal models (Wilensky et al., 2006). Studies have indicated that dopamine transmission in these complexes is key to the formation, retrieval and expression of emotional memory during fear conditioning (de Oliveira et al., 2011; Ehrlich, 2009; Fadok et al., 2010). Several studies on emotional processing have indicated that volume reduction and incorrect activity occur in the amygdalae during emotional stress in patients with schizophrenia (Aleman and Kahn, 2005; Benes, 2010; Shayegan and Stahl, 2005).

Previously, using in vivo microdialysis techniques, we demonstrated that phasic dopamine release in the amygdalae in response to a fear-conditioned cue was excessive in methamphetamine-sensitised rats, suggesting that excessive dopamine release was an endophenotype of vulnerability to psychological stress (Suzuki et al., 2002). Subsequently, we demonstrated that haloperidol increased basal dopamine release in the amygdalae, thereby attenuating excessive phasic dopamine release in response to a

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fear-conditioned cue (Oshibuchi et al., 2009). These results suggest that the modulation of dopamine release is involved in the therapeutic mechanisms of antipsychotic treatment in schizophrenia.

Based on previous reports, we aimed to clarify the mechanisms of the specific benefits of clozapine in schizophrenia treatment. Therefore, we examined the effects of clozapine compared with haloperidol on basal and phasic dopamine release in the basolateral amygdalae of methamphetamine-sensitised rats in response to a fear-conditioned cue. Analysis of the extracellular dopamine level was conducted by in vivo microdialysis and high-performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Animals

We used male Sprague–Dawley rats (CLEA Japan, Inc.) weighing 160–180 g that were subjected to handling for 15 min every 3 days after arrival before undergoing a stereotaxic operation when they weighed 290–350 g. The animals were maintained at a constant room temperature (23 °C ± 2 °C) in a 12-h light–dark cycle (dark from 20:00 h) and had free access to water and food. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Medicine Animal Experiments and Ethics Committee of the Tokyo Women's Medical University School.

2.2. Drugs

Clozapine and haloperidol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Haloperidol was dissolved in 1.0 mg/ml 0.5% lactate and saline, and clozapine was dissolved in 1, 3 or 10 mg/ml 0.05 M hydrochloric acid saline. The acidity of these solutions was adjusted to a pH of 5.0–6.0 using a 1-M solution of NaOH. HCl was dissolved in saline to produce a 0.05-M solution of HCl, which was used as a control.

2.3. Methamphetamine sensitisation

To produce a reverse-tolerance model, methamphetamine was administered to rats using a previously reported method (Oshibuchi et al., 2009). In the methamphetamine group, methamphetamine hydrochloride was dissolved in saline (2 mg/ml) and injected subcutaneously for 10 days at a dosage of 2 mg/kg/day. In the non-sensitised control group, saline was administered in equivalent volumes.

2.4. Fear stress-conditioning protocol

Fear stress conditioning was performed according to previously published methods (Suzuki et al., 2002). Fear conditioning was performed once per day for 3 consecutive days by transferring the rats from their home cages to stimulation cages (clear sided, height: 45 cm, width: 22 cm and depth: 22 cm) in a soundproof room where an electric foot shock was applied using a floor grid comprising stainless steel rods (diameter of 4 mm at 8-mm intervals). A 30-s continuous 80-db sound was used for fear conditioning, and it was emitted before the 2-s administration of a 0.8-mA electric foot shock as the unconditioned stimulus (in the fear-conditioned groups). The electric foot shock was provided as a constant current stimulus that was produced by a shock generator/scrambler (Muromachi Kikai, Tokyo, Japan). Sham conditioning was performed in the control groups, which were exposed to auditory stimulation under the same conditions, but without a foot shock.

2.5. Surgical implantation of cannulas

Under pentobarbital anaesthesia (50 mg/kg i.p.) and by using a stereotaxic frame, a guide cannula was inserted into the left basolateral amygdalae at 2.4 mm posterior and at 5.2 mm lateral to the bregma at a depth of 7.2 mm from the surface of the bone at the bregma (Paxinos and Watson, 1997). An ear bar with dulled tips was used as an anchor to avoid damaging eardrums of the rats. The rats rested and were allowed sufficient recovery for more than 1 day in their individual home cages (opaque sided, height: 30 cm, width: 25 cm and depth: 15 cm).

2.6. Microdialysis

Microdialysis was initiated after 2 days of the operation. A probe was inserted into the left amygdalae on the day before microdialysis. The dialysis probe had a 2.0-mm membrane length, an outer diameter of 0.5 mm, and a molecular weight cut-off of 20000 Da (AI-12–2; Eicom, Kyoto, Japan). The samples were collected at a flow rate of 2 μl/min with Ringer's solution used as a perfusate following acclimation for 180 min after the beginning of microdialysis. First, tonic level samples were collected for 80 min before one of the following was intraperitoneally injected: haloperidol, 1 mg/kg; clozapine, 1, 3 or 10 mg/kg; or 0.05 M HCl dissolved in saline in the same volume used as the control. Second, post-drug samples were collected for 240 min (80–320 min after the start of collection). The fear-conditioned stimulus (sound only, with no foot shock) was then applied to the rats in all groups. Post-conditioned stimulus samples were collected for 80 min (320–400 min after the beginning of correction). The total microdialysis runtime was 580 min (acclimation, 180 min; sampling of tonic levels, 80 min; post-drug samples, 240 min; and post-conditioned stimulus samples, 80 min).

2.7. Measurement of extracellular dopamine levels

Extracellular dopamine levels were measured by HPLC every 20 min The samples were collected with an Auto Injector (ESA-20; Eicom) and placed in an HPLC (HITEC-500: Eicom) every 20 min to quantify the dopamine level in real-time. The HPLC used a CA-5ODS column (2.1 × 150 mm; Eicom) with a mobile phase containing 134.49 g/L Na2HPO4, 49.40 g/L Na2HPO4, 1% methanol, 800 mg/L sodium 1-decanesulfonate and 50 mg/L EDTA-Na2. The detector in this system was equipped with a graphite working electrode set at +0.45 V relative to an Ag/AgCl reference electrode.

2.8. Histology

At the end the study, the animals were given an overdose of sodium pentobarbital (100 mg/kg) and transcardially perfused with physiological saline followed by 10% buffered formalin. The location of the microdialysis probe in the amygdalae was determined histologically by serial coronal sections (50 μm) that were stained with haematoxylin–eosin. We excluded data related to bleeding around the trace of the probe or extending beyond the range of the basolateral amygdalae, including the caudate putamen. Only the data obtained from the rats with correctly implanted probes were included in the results (Fig. 1).

2.9. Grouping for microdialysis

Seventy-two rats were divided into 12 subgroups of six rats each. The four main groups were as follows: (1) methamphetamine-sensitised rats subjected to fear conditioning (MAP.FC), (2) methamphetamine-sensitised rats subjected to sham fear conditioning (MAP.sham), (3) non-sensitised (saline-given) rats