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Valproic acid inhibits excess dopamine release in response to a fear-conditioned stimulus in the basolateral complex of the amygdala of methamphetamine-sensitized rats

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ABSTRACT

Valproic acid, an established antiepileptic and antimanic drug, has recently emerged as a promising emotion-stabilizing agent for patients with psychosis. Although dopamine transmission in the amygdala plays a key role in emotional processing, there has been no direct evidence about how valproic acid acts on the dopaminergic system in the brain during emotional processing. In the present study, we tested the effect of valproic acid on a trait marker of vulnerability to emotional stress in psychosis, which is excess dopamine release in response to a fear-conditioned stimulus (CS) in the basolateral complex of the amygdala of methamphetamine-sensitized rats. Extracellular dopamine was collected from the amygdala of freely moving methamphetamine-sensitized rats by in vivo microdialysis and was measured using high-performance liquid chromatography. During microdialysis, valproic acid was intraperitoneally injected followed by CS exposure. Valproic acid treatment decreased baseline levels of dopamine and also attenuated the excess dopamine release in response to the CS in the amygdala of methamphetamine-sensitized rats. The results prove that valproic acid inhibits spontaneous dopamine release and also attenuates excess dopaminergic signaling in response to emotional stress in the amygdala. These findings suggest that the mechanisms of the emotion-stabilizing effect of valproic acid in psychosis involve modulation of dopaminergic transmission in emotional processing.

1. Introduction

Valproic acid, an established antiepileptic and antimanic drug, has recently emerged as a promising emotion-stabilizing agent for patients with psychosis. Clinical studies have demonstrated that valproic acid is effective and improves emotional reliability in psychosis (Gobbi et al., 2006; Suzuki et al., 2009). However, the mechanisms behind these therapeutic benefits are not fully understood.

To examine emotional processing, fear conditioning, which is based on the classical conditioning theory, has been developed as a form of psychological stress. The basolateral complex of the amygdala is one of the most potent modulators of the mechanisms that are responsible for the emotional memory system in animal models (Simmons et al., 2007). Studies have also determined that the dopamine transmission in the basolateral complex of the amygdala during fear conditioning plays a key role in the formation, retrieval, and expression of emotional memory (Bissière et al., 2003; Fadok et al., 2010; de Oliveira et al., 2011). Previous studies have demonstrated that valproic acid increased the extracellular dopamine levels in the medial prefrontal cortex and hippocampus; this is a mechanism that is believed to improve cognition in patients with schizophrenia (Ichikawa and Meltzer, 1999; Ichikawa et al., 2005; Huang et al., 2006). However, to the best of our knowledge, prior to this study, the effects of valproic acid on extracellular dopamine levels in the amygdala had not been discussed; therefore, the effect of valproic acid on dopamine release in relation to emotional processing had not yet been determined. In a previous study that evaluated psychosis in animal models, a significantly higher level of dopamine release was observed in the amygdala of methamphetamine-sensitized (reverse tolerance) rats following exposure to a fear-conditioned stimulus (CS) than in non-sensitized animals (Suzuki et al., 2002). Furthermore, in a previous research we also demonstrated that antipsychotic agents attenuated dopamine release in the amygdala in response to a CS (Oshibuchi et al., 2009). These results indicate that the excess dopamine release in the amygdala of methamphetamine-sensitized rats in response to the CS is considered as a valid trait marker when evaluating the effects of a drug on dopaminergic hypersensitivity to salient stimuli in patients with psychosis and their vulnerability to emotional stress.

In the present study, to investigate the pharmacological effects of valproic acid on the emotional component of psychosis, we first

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examined the physiological and pharmacological effects of valproic acid on spontaneous dopamine release in the basolateral complex of the amygdala of naïve Sprague-Dawley rats. We then examined the effects of valproic acid on dopamine release after the CS in the basolateral complex of the amygdala of methamphetamine-sensitized and fear-conditioned rats using in vivo microdialysis and high performance liquid chromatography (HPLC) techniques.

2. Materials and methods

2.1. Animals

In this study, we used male Sprague-Dawley rats (Japan CLEA, Tokyo, Japan) that weighed180–190 g upon arrival at the laboratory. The rats were then subjected to handling for 15 min every three days. They weighed 328–390 g at the time of stereotaxic surgeries. Animals were maintained at constant room temperature (26 ± 2 °C) in a 12 h/12 h light/dark cycle (darkness was maintained from 20:00 h) environment with free access to water and food. All procedures were performed with minimal pain in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals. The Animal Experiments and Ethics Committee of the Tokyo Women's Medical University School of Medicine approved all procedures.

2.2. Drugs

Sodium valproate was kindly donated by Kyowa Hakko Kirin Co., Ltd. (Tokyo, Japan). All other chemicals and reagents used were the purest commercially available and were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium valproate was dissolved at 30 or 100 mg/mL in physiological saline. Methamphetamine hydrochloride was purchased from Dainippon Sumitomo Pharmaceutical (Osaka, Japan) and was dissolved at 2 mg/mL in physiological saline.

2.3. Methamphetamine sensitization

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

2.4. Surgical implantation of cannulae

Using a previously reported method, following sensitization, a guide cannula was inserted using a stereotaxic frame (Model 900, David Kopf Instruments, California, USA) into the left basolateral complex of the amygdala at a point 2.4-mm posterior and 5.2-mm lateral to the bregma and at a depth of 7.2 mm from the surface of the bone at the bregma (Paxinos and Watson, 1986). Pentobarbital anesthesia (50 mg/kg; mean body weight at surgery, 354.3 g) was used during the procedure. To avoid damage to the rats' eardrums during the stereotaxic procedure, an ear bar with dulled tips was used as an anchor. After surgery, the rats were transferred to their individual home cages (opaque-sided: 30-cm high, 25-cm wide, and 15-cm deep) to avoid damage to the guide cannula.

2.5. Fear conditioning protocol

When the rats were 8–10 weeks of age, fear conditioning (cue and electrical foot shock) was performed for three consecutive days, beginning two days following the surgery until recovery. In the fear-conditioned group (FC), a continuous sound of 80 dB for 30 s was used as a CS, which was emitted 2 s before the administration of the electric foot shock (i.e., unconditioned stimulus) at 2 mA for 2 s. The electric foot shock was a constant current stimulus produced by a shock generator/scrambler (Muromachi Kikai, Tokyo, Japan). Sham conditioning was performed in the control group that was exposed to auditory stimulation under the same conditions but with no foot shock.

2.6. Microdialysis

Microdialysis in awake, freely moving rats was initiated 2–4 days after the fear conditioning. A probe was inserted into the left amygdala a day before the microdialysis was started. The dialysis probe had a membrane length of 2.0 mm, an outer diameter of 0.5 mm, and a molecular weight cut-off of 20,000 Da (AI-12-2; Eicom, Kyoto, Japan). Ringer solution (Na^+, 147 mM; K^+, 4 mM; Ca^{2+}, 2.3 mM; and Cl^-, 155.6 mM) was used as the perfusate for the microdialysis, and samples were collected at a flow rate of 2 μL/min. Samples were collected following acclimation for 180 min after the beginning of microdialysis. First, baseline level samples were collected for 80 min; a drug (valproic acid 30 mg/kg, valproic acid 100 mg/kg, or saline control 1 mL/kg as same volume with valproic acid treatment) was then intraperitoneally injected. Second, post-drug samples were collected for 60 min. The CS (sound only, with no foot shock) was then applied to rats in all groups. After the CS exposure, samples were then collected for 80 min. The total microdialysis run time was 400 min (acclimation, 180 min; sampling of baseline levels, 80 min; post-drug samples, 60 min; and after CS samples, 80 min).

2.7. Measurement of extracellular dopamine levels

Samples were collected every 20 min and the extracellular dopamine levels (in pg) were measured in each sample (40 μL per 20 min) using HPLC (HITEC-500: Eicom). During HPLC, a CA-5ODS column (2.1×150 mm; Eicom) with a mobile phase (containing NaH_2PO_4, 134.49 g/L; Na_2HPO_4, 49.40 g/L; methanol, 1%; sodium 1-decanesulfonate, 800 mg/L; and EDTA-2 Na, 50 mg/L) was used. The detector used 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 of each experiment, the rats were given an overdose of sodium pentobarbital (100 mg/kg) and were perfused transcardially with physiological saline followed by 10% buffered formalin. The location of the microdialysis probe in the basolateral complex of the amygdala was determined histologically using serial coronal sections (50 μm) stained with hematoxylin and eosin. Only the data obtained from rats with correctly implanted probes were included in the results (Fig. 1). Data that clearly represented preparations with bleeding around the tip of the probe or preparations from incorrectly implanted probes extending beyond the range of the basolateral complex of the amygdala or that included the caudate putamen were excluded.