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Seizure-induced 5-HT release and chronic impairment of serotonergic function in rats

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HIGHLIGHTS

- Seizure induced 5-HT release at acute period.
- Pilocarpine injection led to drastic lost of tryptophan hydroxylase (TPH) in raphe nuclei.
- The decrease of 5-HT may due to the impairment of serotonergic function in raphe nuclei.

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ABSTRACT

We analyzed the dynamic concentration change of serotonin (5-HT) and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) within the epileptic hippocampus in rats. Seizure was induced by systemic injection of pilocarpine (320 mg/kg, i.p.). Using electroencephalography (EEG) recordings, we found that primary seizure discharge was induced 30 min after pilocarpine administration and that recurrent discharge peaked 14d after the onset of status epilepticus (SE). The extracellular fluid in the hippocampus was sampled by microdialysis from conscious animals at various time points before and after SE. The concentrations of 5-HT and 5-HIAA in the samples were measured by high-performance liquid chromatography and electrochemical detection (HPLC-ECD). Interestingly, 5-HT levels in the hippocampus were dramatically increased within the 30 min following SE. This reversed to basal level by 4d after SE and continued to drop to 48% at 7d and 28% of basal level 14d after SE. Accordingly, a marked increase of 5-HIAA in the hippocampus appeared at 2d after SE, then gradually declined to levels below baseline. To identify serotonergic neurons in the raphe nuclei (a major source of 5-HT release in the brain), brain sections were immunostained for tryptophan hydroxylase (TPH). The number of TPH positive neurons and the intensity of TPH staining significantly decreased at 28d after SE. These data suggest that pilocarpine induces depletion of 5-HT in the hippocampus and significantly compromise serotonergic neurons in the raphe nuclei. The loss of serotonergic function may play a significant role in the pathophysiology of epilepsy.

1. Introduction

Increasing evidence suggests that serotonergic neurotransmission is involved in a variety of experimental animal models of seizure and its change may account for increased seizure susceptibility in genetically modified rodents [8,9]. Despite intensive studies, however, the role of 5-HT in epilepsy remains controversial. Previous work has shown that 5-HT has both convulsive and anticonvulsive effects, for example, Choi et al. [10] found that treatment with selective serotonin reuptake inhibitor (SSRI) may increase seizure susceptibility in normal rats and increase the frequency and intensity of seizing in epileptic rats. However, Smolders et al. [11] reported that agents that elevate extracellular (EC) serotonin (5-HT) levels, such as 5-hydroxytryptophan (5-HTP) and serotonin reuptake inhibitors, suppressed both focal and generalized seizures. In addition, depletion of 5-HT in the brain lowers the threshold to audiogenically, chemically and electrically evoked convulsions [1,4].

Research investigating the effects of acute seizure on the concentration of 5-HT in the brain also yielded conflicting results

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[2,7,20]. In order to clarify the effect of seizure on serotonin level in the brain, we studied the dynamic change of serotonin in the hippocampus during acute and chronic periods of epilepsy.

2. Methods and materials

Two-month-old male Sprague–Dawley (SD) rats (220–250 g) were housed under a 12 h/12 h light/dark cycle and had ad libitum access to food and water. All animal experiments were performed according to the Guidance for Animal Experimentation of Fujian Medical University.

Animals were divided into two groups. For the control group (CTRL), 8 rats received 0.9% saline IP injection. For the pilocarpine group (PILO), 15 rats were IP injected with pilocarpine hydrochloride (320 mg/kg) 30 min after atropine (1 mg/kg, i.p.) administration. The use of atropine was to block the peripheral cholinergic effects of pilocarpine. After the drug administration, the progressive evolution of seizing behavior was observed and scaled according to the Racine score [19]. Only those animals that developed stage IV–V seizure were used. Status Epilepticus (SE) was defined as continuous stage IV–V seizure for more than 30 min. For electroencephalography (EEG) recording, rats were implanted with an electrode using the following coordinates: AP 2.5 mm, ML 2.0 mm and DV 0.5 mm. A ground electrode was inserted into the occiput and a reference electrode placed subcutaneously. Animal behavior and EEG were monitored for two continuous hours by a video monitoring system (KT-88-2400, Chengdu, China) three times per day in the following 28 days after pilocarpine application. EEG discharges with amplitudes exceeding 50 μV, a value typical of 3× of basal level EEG amplitude, were counted as seizure discharges.

For microdialysis, animals were anesthetized with 10% chloral hydrate (i.p., 2 ml/kg) and placed on a stereotaxic frame (51600, Stoelting, USA). The microdialysis probes (CMA 12, Solna, Sweden) were unilaterally implanted into the hippocampal CA3 region using the following coordinates: AP 4.8 mm, ML 5.0 mm and DV 4.6 mm. After the operation, rats were housed individually and given Gentamicin (80,000 IU) every day for 7 days to prevent infection. Three days after the implantation, the inlets of the microdialysis probes were attached to a microinfusion pump (CMA/102, Stockholm, Sweden) and the outlets were attached to the cooling collector (CAM170, Stockholm, Sweden). Rats were put in a plexyglass cage (42 cm × 42 cm × 20 cm) unstrained. Concentric microdialysis probes (CMA 12, Solna, Sweden, outer membrane diameter: 0.5 mm, membrane length: 4 mm and molecular weight cut-off: 20 kDa) were perfused with artificial cerebrospinal fluid (ACSF) containing the following (in mM): 145 NaCl, 3.8 KCl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 25 $NaHCO_3$, 5.4 glucose, bubbled with 95% O_2/5%CO_2, pH 7.4. The purfusion was carried out at a flow rate of 2 μl/min via a 250 μl Hamilton syringe connected to a microinfusion pump. After an equilibration period of 2 h, the first three fractions were collected to obtain the basal value and the following one out of every three fractions were collected for the following groups: Before SE (pre-SE), 30 min after SE (SE 0d), 2 days after SE (SE 2d), 4 days after SE (SIL 4d), 7 days after SE (SIL 7d), and 14 days after SE (CHRN 14d).

The dialysates were kept at −80 °C or immediately separated and detected by a Breeze HPLC system with electrochemical detector from Waters (Milford, MA, USA), the detector was connected to a glass carbon electrode (Ag/AgCl reference) with the voltage set at 0.7 V. The HPLC column used was a C18 reversed phase column (Grace, USA). The mobile phase consisted of 120 mM NaH_2PO_4, 250 mg/L sodium heptanesulfonate, 80 mg/L EDTA, and 16% (vol/vol) methanol (final pH 3.2, flow rate 0.9 ml/min).

At the end of the experiment, animals were transcardially perfused under deep anesthesia (10%chloral hydrate, 2 ml/kg) with 0.1 M phosphate-buffered Saline (pH 7.4) followed by 4% paraformaldehyde. Coronal brain sections (20 μm thick) were processed by H&E staining for histological verification of the localization of the microdialysis probe. Only data from rats with correct probe placement were used for further analysis. Meanwhile, the brain slices (5 μm thick) containing raphe nuclei were prepared and immunostained for tryptophan hydroxylase (TPH, mouse monoclonal, T1299 from Sigma, 1:1000 dilution), visualized by the SP kit to identify serotonergic neurons. Stained brain sections were observed using a Leica-DM2500 microscope (Leica, Germany). Images were captured using a digital camera and the Leica

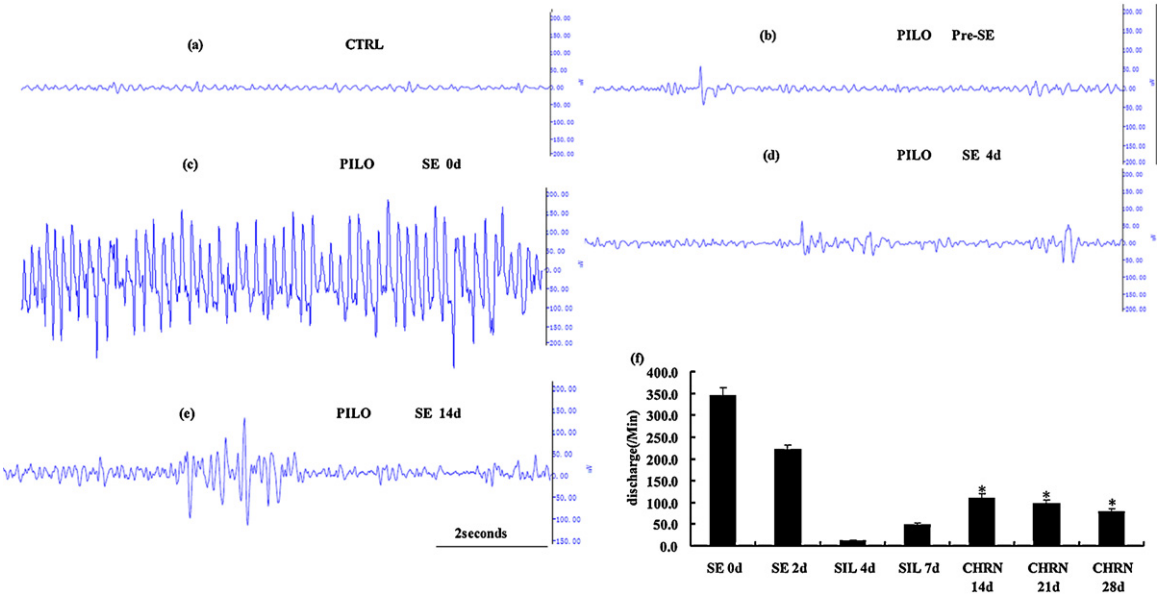

Fig. 1. Pilocarpine injection induced epileptic discharges in the electroencephalography (EEG) recording in rats. (a–e) Representative EEG recording in a different period of epilepsy: (a) CTRL and (b) PILO pre-SE rats displaying α-wave. (c) PILO SE 0d rats showing continuous intensive spike-wave, (d) at SIL 4d, α-wave was accompanied by a few low sharp-waves or spike-and-slow-wave complex. (e) At CHRN 14d, mixed display of pike-wave, spike-and-slow-wave complex, sharp-waves and multiple spike-and-slow-wave complex. (f) Time course of seizure discharge after pilocarpine injection. Statistical difference was found between SIL 7d and CHRN 14d, CHRN 21d, and CHRN 28d (*$p < 0.01$, $n = 10$).