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Effects of meclofenamic acid on limbic epileptogenesis in mice kindling models

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HIGHLIGHTS

- MFA retarded kindling acquisition in amygdaloid kindling model.
- MFA prevented post-kindling enhanced amygdala excitability.
- MFA decelerated the development of hippocampus rapid kindling epileptogenesis.

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ABSTRACT

The most avid goal for antiepileptic drugs (AEDs) development today is to discover potential agents to prevent epilepsy or slow the process of epileptogenesis. Accumulating evidence reveals that gap junctions in the brain may be involved in epileptogenesis. Meclofenamic acid (MFA), a gap junction blocker, has not yet been applied in epileptogenic models to test whether it has antiepileptogenic or disease-modifying properties or not. In this study, we investigated the effects of MFA on limbic epileptogenesis in amygdaloid kindling and hippocampus rapid kindling models in mice. We found that intracerebroventricular (*i.c.v.*, 2 μl) administration of either dose of MFA (100 μM, 1 mM or 100 mM) 15 min prior daily kindling stimulus decreased seizure stage, shortened the after-discharge duration (ADD) and increased the number of stimulations required to elicit stage 5 seizure. MFA also prevented the establishment of post-kindling enhanced amygdala excitability, evident as the increase of afterdischarge threshold (ADT) compared with pre-kindling values. Furthermore, MFA retarded kindling acquisition in mice hippocampus rapid kindling model as well, which demonstrated that the antiepileptogenic effects of MFA were not specific to the amygdala but also occur in other limbic structures such as the hippocampus. Our results confirm that MFA can slow the limbic epileptogenesis in both amygdaloid kindling and hippocampus rapid kindling models, and indicate that MFA may be a potential drug that has antiepileptogenic or disease-modifying properties.

1. Introduction

Epileptogenesis, broadly defined as the transformation of the normal brain to a chronic epileptic state, bears complex cascades of morphological and functional changes. Currently, most antiepileptic drugs (AEDs) available are only symptomatic treatments that suppress seizures and few of them have antiepileptogenic or disease-modifying properties that prevent or delay the development of epileptic seizures [11,12]. These phenomena indicate that mechanisms underlying epileptogenesis and ictogenesis probably differ. Thus, it is rational to anticipate that potential antiepileptogenesis drugs possibly have different targets of action other than those of traditional AEDs.

Gap junctions, composed of connexin (Cx) subunits, are intercellular membrane channels that provide direct cytoplasmic continuity between adjacent cells and allow propagation of electrical impulses and exchange of small molecules [7]. An increasing number of studies suggest that gap junctions in the brain may be involved in the process of epileptogenesis [7,19,23,26]. Synchronous neuronal networks connected by gap junctions are important in the generation of high-frequency oscillations (HFOs), which are an indication of an epileptiform network mechanism in epileptogenesis [23]. Astrocytic gap junctions can influence the balance of neuronal microenvironments by buffering extracellular K^+ and delivering energetic metabolites, which in turn affect neuron functions during seizure events [19,26]. Changes of connexins

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mRNA and protein are also observed in limbic regions in electrical kindling and post-status epilepticus models [7]. Thereby gap junctions may be a potential drug target to interfere epileptogenesis.

Meclofenamic acid (MFA) is a fenamate that has also been used to block gap junction channels through both astrocytic Cx43 [6,21,30] and neuronal Cx36 [3,25]. Although MFA can inhibit seizures in tetanus toxin-induced refractory focal cortical epilepsy in rats [14], there are no further studies to test whether or not MFA has antiepileptogenic or disease-modifying effects in epileptogenic models. Electrical kindling is the most widely used model of epileptogenesis in which repeated initial subconvulsive stimulation triggers progressive intensification of behavioral and electrographic seizure activity and the kindling period provides a means of testing putative antiepileptogenic agents [10]. In the present study, we sought to investigate the effects of MFA on limbic epileptogenesis in mice amygdaloid kindling and hippocampus rapid kindling models.

2. Materials and methods

2.1. Animals

The animals used in this study are male C56BL/6 (22–25 g, Experimental Animal Center, Zhejiang Academy of Medical Science, Hangzhou, China), maintained in individual cages with a 12 h light–dark cycle (lights on from 8:00 to 20:00). Water and food were given ad libitum. Experiments were carried out each day between 10:00 and 17:00. All Experiments were approved by and conducted in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize any pain or discomfort, and the minimum number of animals was used.

2.2. Surgery

Under sodium pentobarbital anesthesia (60 mg/kg, *i.p.*), mice were fixed in a stereotaxic apparatus (512600, Stoelting, USA), and electrodes were implanted in the right basolateral amygdala (coordinates in mm from bregma, AP: −1.8, *L*: −3.0, *V*: −4.8) or in the right ventral hippocampus (AP: −2.9, *L*: −3.0, *V*: −3.0) based on the Franklin and Paxinos (1997) atlas [5]. The electrodes were made of two twisted stainless steel Teflon-coated wires (diameter 0.125 mm, maximal tip separation 0.5 mm, A.M. Systems, USA) insulated except for 0.5 mm at the tip. Electrodes were connected to a miniature receptacle, which was attached to small screws placed in the skull with dental cement. Apart from electrodes implantation, a guide cannula (62003, RWD Life Science Co., Ltd) was stereotaxically implanted into the right lateral ventricle (AP: −0.5, *L*: −1.0, *V*: −2.2) of mice for daily drug administration. At least one week was allowed for recovery from surgery.

2.3. Kindling procedure

Electrical stimulations were delivered by a constant-current stimulator (SEN-7203, SS-202J, Nihon Kohden, Japan) and electroencephalograms (EEGs) were recorded with a Neuroscan system (Compumedics, Melbourne, Australia). As previous description [27,29], the afterdischarge threshold (ADT) was determined by an application of a 1 s train of 1 ms monophasic square-waves at 60 Hz, beginning at 40 μA and increasing by 20 μA which were given at 30 min intervals until an electrographic afterdischarge (AD) lasting at least 5 s was elicited. Amygdaloid kindling stimulations (1 ms pulses, 60 Hz frequency, 1 s duration) were applied following standard protocol consisting of once-daily stimulation at ADT. The day of ADT determination was considered day 0 of kindling. Stimulations were delivered daily for 15 days.

The hippocampus rapid kindling procedure was similar to the conventional kindling described above except that six kindling stimulations at 30 min intervals with an intensity of 100 μA was delivered for six consecutive days [13,18].

Behavioral seizures were scored according to Racine's scale [17], as modified for the mouse: stage 0, no response or behavior arrest; stage 1, chewing or facial twitches; stage 2, chewing and head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling. Seizure stages 1 and 2 indicate focal seizures, while stages 3–5 are considered as generalized seizures [4]. When mice exhibited three consecutive stage 5 seizures, they were regarded as fully kindled. In addition to seizure stage, the AD duration (ADD) was also recorded.

2.4. Evaluation of the effects of MFA on limbic kindling acquisition

Twenty-four hours after determination of ADT, mice were divided into four groups to receive intracerebroventricular (*i.c.v.*) infusion of saline or different doses of MFA (M4531 Sigma, 100 μM, 1 mM and 100 mM dissolved in sterile saline) 15 min prior the amygdaloid kindling stimulation for 15 consecutive days. Saline or MFA were infused (2 μl in 3 min) via an internals cannula, which extended 0.5 mm below the tip of the guide cannula. Flow rate was controlled by a microsyringe pump (World Precision Instruments Inc.). The internals cannula was left in place for 5 min and then removed, and the guide was sealed with a cap. For testing the effects of MFA on hippocampus rapid kindling, saline and the same doses of MFA were given 15 min prior first kindling stimulation for six consecutive days. One week after the last kindling stimulation of either kindling procedure, ADT of mice of different groups were tested again.

2.5. Histology

At the end of all the experiments, electrode and guide cannula placements were histologically verified. Brain sections were cut (10 μm) and stained with toluidine blue O. Only mice with electrodes lying within the right basolateral amygdala or right ventral hippocampus and guide cannula within the right lateral ventricle were included in the statistical analysis. In our experiments, 75 out of 110 mice had electrodes and guide cannulas correctly located in both targets.

2.6. Statistical analysis

Data were analyzed using PASW Statistics 18.0 for Windows. Analysis of group differences in kindling acquisition was performed by two-way analysis of variance (ANOVA) for repeated measures with Fisher's least significant difference (LSD). Comparisons of the number of stimulations required in and to each seizure stage during kindling acquisition were made with one-way ANOVA followed by the LSD test. One-way ANOVA with LSD test was also used for analyzing effects of MFA on change of ADT properties in mice after kindling. In the case of comparing the incidence of focal or generalized seizures, the χ^2 test was used. Data are presented as mean ± SEM. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. MFA retarded amygdaloid kindling acquisition

To determine the effects of different doses of MFA on epileptogenesis, we used progression of behavioral seizures, electrographic