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UNILATERAL LOW-FREQUENCY STIMULATION OF CENTRAL PIRIFORM CORTEX DELAYS SEIZURE DEVELOPMENT INDUCED BY AMYGDALOID KINDLING IN RATS

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Abstract—Low-frequency stimulation of the kindling site interferes with the course of kindling epileptogenesis. The present study examined the effect of unilateral low-frequency stimulation of the central piriform cortex on seizure development induced by amygdaloid kindling in rats. The ipsilateral or contralateral central piriform cortex received low-frequency stimulation (15 min train of 0.1 ms pulses at 1 Hz and 50–150 μA) immediately after termination of once daily kindling stimulation (2 s train of 1 ms pulses at 60 Hz and 150–300 μA) in the right amygdala for 30 days. Low-frequency stimulation of either the ipsilateral or contralateral central piriform cortex significantly suppressed the progression of seizure stages and reduced afterdischarge duration throughout the course of amygdaloid kindling. The marked suppression induced by low-frequency stimulation of the central piriform cortex on either side was predominantly due to the significant retardation of progression from stage 0 to stage 1 and stage 3 to stage 4 seizures. In addition, the suppressive effect of low-frequency stimulation did not disappear when the stimulation was stopped; it could persist for at least 10 days. These findings indicate that brain areas other than the kindling focus, such as the central piriform cortex on both sides, can also be used as reasonable targets for low-frequency stimulation to retard seizure development induced by amygdaloid kindling. Secondly, like the ipsilateral central piriform cortex, the contralateral central piriform cortex may also participate in the progression and secondary generalization of focal seizures. The study suggests that unilateral low-frequency stimulation of the central piriform cortex may have a significant antiepileptogenic effect, and may be helpful for exploring effective and long-lasting therapies for human temporal lobe epilepsy. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: epilepsy, focal seizure, generalized seizure, long-term depression.

Electrical stimulation for the treatment of pharmacoresistant epilepsies is now being widely studied both clinically and experimentally (Velasco et al., 2001; Richardarson et al., 2003; Goodman et al., 2005). Low-frequency stimulation (LFS, 1–3 Hz), similar to that which induces long-term depression (LTD) *in vitro* (Fujii et al., 1991), has been demonstrated to have a profound and long-lasting protective effect on epileptic activity (Gaito, 1980a,b; Gaito and Gaito, 1980; Weiss et al., 1995). LFS of the kindling focus induces an increase in afterdischarge (AD) threshold in hippocampal and amygdaloid kindling, and these effects persist for 5–12 days (Ullal et al., 1989). LFS at the focus also significantly delays the seizure development induced by amygdaloid kindling in rats (Weiss et al., 1998; Velisek et al., 2002). Recently, Goodman et al. (2005) reported a dramatic decrease in the incidence of stage 5 seizures in fully kindled animals after preemptive LFS at the kindling focus. However, in these studies the LFS was always applied at the kindling focus, and no data have been presented examining whether LFS at other brain areas can also produce an inhibitory effect against kindling seizures in animals.

The piriform cortex (PC) is the largest area of the mammalian olfactory cortex and has extensive connections to and from other limbic nuclei. To date, a number of studies have demonstrated that the PC is part of an epileptogenic network and plays a critical role in the development and maintenance of limbic kindling and other types of limbic epileptogenesis leading to complex partial seizures (Löscher and Ebert, 1996). Moreover, in contrast to the anterior and posterior PC, the central piriform cortex (cPC) may have preferred access, either directly or indirectly, to structures capable of supporting generalized kindled seizures (Schwabe et al., 2004b; Katarzyna et al., 2004). However, most previous studies investigated the role of the PC bilaterally, including bilateral injections of ibotenate, vigabatrin and 2-amino-5-phosphonovaleric acid (Morimoto et al., 1986; Stevens et al., 1988; Schwabe et al., 2000, 2004a,b), while few studies have directly investigated the role of the ipsilateral and contralateral PC in kindling seizures. Thus, it remains unclear whether unilateral treatment of the PC has effects similar to those seen bilaterally. Furthermore, the roles the ipsilateral and contralateral PC play in epileptic activity may differ. Lehmann et al. (1998) reported that amygdaloid kindling leads to a significant decrease in the number of GABAergic interneurons in the ipsilateral PC but not the contralateral side.

Therefore, the present study was designed to investigate the effect of unilateral LFS of the cPC on seizure development induced by amygdaloid kindling in rats, which is a widely used model of complex partial epilepsy with secondary generalization (Albright and Burnham, 1980).

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Abbreviations: AD, afterdischarge; cPC, central piriform cortex; EEG, electroencephalogram; LFS, low-frequency stimulation; LTD, long-term depression; PC, piriform cortex.

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Furthermore, the roles of the ipsilateral and contralateral PC in amygdaloid kindling were directly investigated.

EXPERIMENTAL PROCEDURES

Animals

All experiments were carried out 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. Furthermore, attempts were made to minimize the number of animals used in the study and their suffering. The animals used in this study were male Sprague–Dawley rats (220–300 g, Grade II, Certificate No. 22-9601018, Experimental Animal Center, Zhejiang University, Hangzhou, China), maintained in individual cages with a 12-h light/dark cycle (lights on from 8:00–20:00 h). Water and food were given *ad libitum*. Experiments were carried out each day between 10:00–17:00 h.

Surgery

Under sodium pentobarbital anesthesia (35 mg/kg, i.p., Abbott, North Chicago, IL, USA), rats were fixed in a stereotaxic apparatus (Narishige, SR-5, Tokyo, Japan), and two electrodes (0.2 mm in diameter) were implanted into the right basolateral amygdala (AP −2.8, L −4.8, V −8.8) and the right (n=18) or left (n=18) central PC (AP −0.8, L ±4.9, V −9.0). All coordinates were measured in mm from bregma according to the atlas of Paxinos and Watson (1998). The electrodes were bipolar twisted stainless steel Teflon-coated wires (tip distance 0.5–1.0 mm, A.M. Systems. Inc., Sequim, WA, USA) and insulated except for 0.5 mm at the tip. Electrodes were connected to a miniature receptacle, which was embedded in the skull with dental cement. At least 10 days were allowed for recovery from surgery.

Kindling and LFS

The electrical stimulation thresholds of the basolateral amygdala and cPC were respectively determined by a 2 s, 60 Hz, 1 ms per pulse and a 1 Hz, 0.1 ms per pulse monophasic square wave stimulus a constant electric stimulator (SEN-7203, SS-202J: Nihon Kohden, Tokyo, Japan). The stimulus intensity began with 50 μA, and was subsequently increased by 20 μA step. Each measurement was separated by at least 30 min in order to insure reliable threshold measurement. For the amygdala, the minimum intensity sufficient to induce AD for at least 5 s was designated as the AD threshold, and used for daily stimulation. The AD threshold intensity ranged from 100 to 300 μA. For the cPC, threshold was established by the appearance of licking or circling behavior. The cPC threshold intensity used for daily LFS ranged from 50 to 150 μA. In the experiments, 36 rats with implanted electrodes were assigned to three groups: controls, LFS of ipsilateral cPC, and LFS of contralateral cPC, matched for AD threshold. The data from six rats with ipsilateral and six with contralateral cPC electrodes were combined as the control group, since they did not differ in the kindling procedure.

Twenty-four hours after the threshold was obtained, the amygdala was subjected to once daily kindling stimulation and the electroencephalogram (EEGs) was recorded through the amygdala electrodes with a PowerLab system (AD Instruments, Sydney, NSW, Australia). In the groups with LFS of the cPC, immediately after cessation of the kindling stimulation in the amygdala, the cPC was subjected to LFS for 15 min. Control rats were also connected to the low-frequency stimulator for 15 min, but no current was delivered. All rats were stimulated for 30 days.

Seizure severity was classified by Racine (1972): (1) facial movement; (2) head nodding; (3) unilateral forelimb clonus; (4) bilateral forelimb clonus and rearing; and (5) bilateral forelimb clonus, rearing and falling. Seizure stages 1–3 indicate complex focal seizures, while stages 4–5 are generalized seizures (Sato et al., 1990). AD duration was also recorded. When the animals exhibited five consecutive stage 5 seizures, they were regarded as fully kindled.

Continued kindling without LFS

After the 30 days' kindling, LFS was suspended. From the 31st day, rats still in the focal seizure stage were kindled for another 10 days. Then the rats rested for 40 days and were kindled again using the initial stimulation parameters.

Histology

At the end of the experiments, electrode placements were histologically verified. Only animals with electrodes lying within the basolateral amygdala and cPC were included in the following analysis.

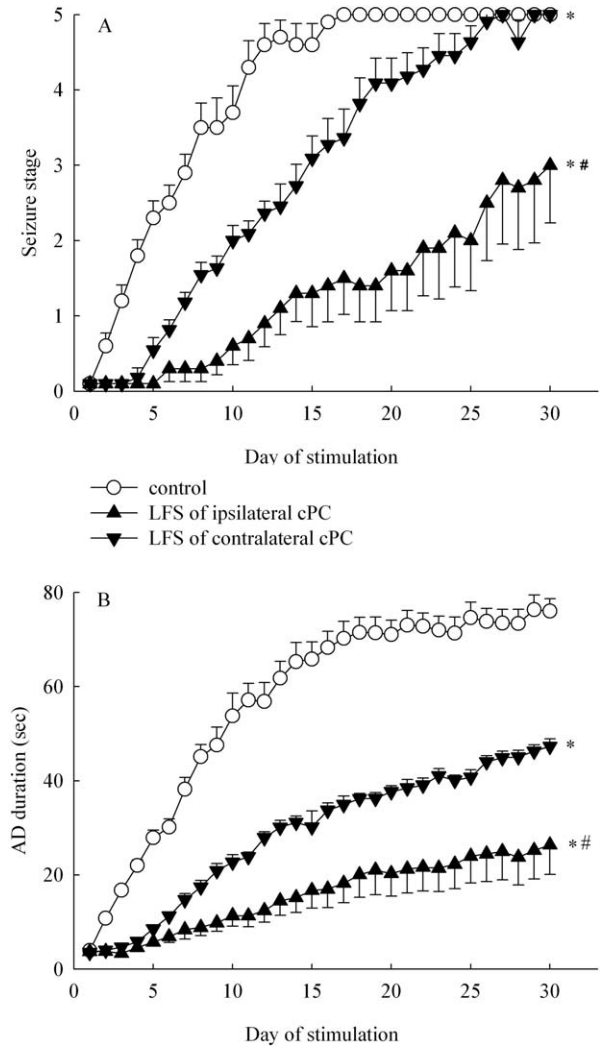

Fig. 1. (A) The effects of LFS of the ipsilateral and contralateral cPC on behavioral stage of seizures during kindling acquisition. (B) Effect of LFS of the ipsilateral and contralateral cPC on AD duration during kindling acquisition. Data are shown as means±S.E.M. * $P<0.0001$ represents statistically significant difference as compared with controls. # $P<0.0001$ represents statistically significant difference as compared with the group with LFS of the contralateral cPC.