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Separating kindling and LTP: Lessons from studies of PKMzeta in developing and adult rats

James G. Heida^{1,*}, Dario J. Englot⁴, Todd C. Sacktor⁵, Hal Blumenfeld⁴, and Solomon L. Moshé^{1,2,3}

¹ Saul R. Korey Department of Neurology, Laboratory of Developmental Epilepsy, The Montefiore/Einstein Epilepsy Center, Albert Einstein College of Medicine, Bronx, NY USA

² Dominick P. Purpura Department of Neuroscience, Laboratory of Developmental Epilepsy, The Montefiore/Einstein Epilepsy Center, Albert Einstein College of Medicine, Bronx, NY USA

³ Department of Pediatrics, Laboratory of Developmental Epilepsy, The Montefiore/Einstein Epilepsy Center, Albert Einstein College of Medicine, Bronx, NY USA

⁴ Department of Neurology, Yale University, New Haven, CT USA

⁵ The Robert F. Furchgott Center for Neural and Behavioral Science, Departments of Physiology, Pharmacology, and Neurology, SUNY Downstate Medical Center, Brooklyn, NY USA

Abstract

The kindling model of temporal lobe epilepsy and the memory model of long-term potentiation (LTP) may have common underlying mechanisms. This is evident by the demonstration that certain signaling molecules play a key role in both. Recently, a brain specific isoform of protein kinase C (PKM ζ) has been shown to play a significant role in both maintaining LTP and memory storage. We were interested in determining if this kinase had a crossover role in kindling-induced epileptogenesis. Using developing and adult rats we examined the role of PKM ζ in kindling. In developing (P15) rats we determined the effect of PKM ζ on retention of amygdala kindling and kindling rate by intra-amygdala administration of a selective PKM ζ antagonist, ZIP (10 nmol). In adult rats we examined the effect of PKM ζ inhibition, ZIP (10 nmol), on afterdischarge (AD) thresholds and kindling retention using rapid hippocampal kindling. Inhibition of PKM ζ by the antagonist ZIP did not affect kindling rate or retention in developing rats. In addition there was also no observed effect on AD thresholds and kindling retention in adult rats. Our results show that, despite the similarities between kindling and LTP in their induction, there is dissociation in the role that PKM ζ plays within the two in maintenance. This may be of importance in establishing a separation between the pathophysiological processes involved in sustaining kindling and the physiological mechanisms involved in maintaining LTP and memory storage.

Keywords

seizures; protein kinase; rat; development

*Corresponding author current address: James G. Heida PhD, Department of Surgery, Division of Neurosurgery, Texas A&M Health Science Center and Neuroscience Institute Scott & White Hospital, 1901 South 1st Street, Bldg 205, Temple TX USA, 76704, Ph: (254) 743-3225, Fax: (254) 742-7482, Email: E-mail: heida@medicine.tamhsc.edu.

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INTRODUCTION

Kindling is a model of complex partial seizures with secondary generalization that results from repetitive, high-frequency electrical stimulation of limbic brain regions, such as the hippocampus and amygdala [4,7,16,17,20]. This model has been widely used to study the basic mechanisms involved in the epileptogenesis of temporal lobe epilepsy (TLE) and has often been used to evaluate the efficacy of antiepileptic drugs [11]. The response of the brain to kindling is that of progressively worsening behavioral seizures with increased duration afterdischarges (AD) recorded on the EEG [7,10,20]. This is the result of the recruitment of vast brain areas outside the initial seizure focus which become reinforced through daily stimulations that ultimately result in generalized seizures. The recruitment and reinforcement in these networks is reminiscent of the plasticity that can occur in long-term potentiation (LTP) [2,22].

LTP is a form of synaptic strengthening that results from high-frequency stimulation in certain brain regions, most notably the hippocampus [3]. Many authors have proposed that LTP is the underlying mechanism by which organisms learn and form memories [19,26,27]. Since LTP was first described by Bliss and colleagues in the 1970's there has been much study of the mechanisms involved in LTP. Over the past 30 years there have been numerous molecules that have been implicated in the underlying synaptic strengthening that occurs with LTP; however, not all of these have been shown to influence memory [15], and those that do involve the initial induction phase of LTP and the early consolidation phase of memory formation. More recently a new molecule implicated in the persistent maintenance of LTP and the storage of memory has been described [19] [21,25]. This molecule is a brain-specific isoform of the protein kinase C, protein kinase M zeta (PKM ζ) [6,21,24]. Blockade of PKM ζ by a selective pseudosubstrate inhibitor ZIP rapidly abolishes preestablished LTP both in hippocampal slices and *in vivo* and the storage of active place avoidance in the hippocampus and conditioned taste aversion memory in the insular cortex, in rats, for a period lasting up to one month. These results show that persistent protein kinase activity may play a crucial role in memory formation and maintenance for long periods of time [25].

Since LTP is considered to be the mechanism by which learning and memory are achieved, and is maintained by PKM ζ , we were interested in whether this kinase also plays a role in maintaining the synaptic alteration of kindling. In order to determine if PKM ζ activity influenced kindling we used both developing and adult male rats. The use of both developing and adult animals would allow us to determine if there were any age-related differences in PKM ζ activity and the consolidation of kindling. Furthermore, since developmental kindling is rapid (single day) it also would allow us to examine if PKM ζ can affect kindling rate. Thus we used PKM ζ inhibition, by intracerebral ZIP administration to the kindling site, and determined its effect on kindling retention (developing and adult rats), AD thresholds (adult rats) and kindling rate (developing rats).

METHODS

Developing Rats

Animals and Surgery—Male rat pups from timed pregnant Sprague-Dawley rats (Taconic Farms Germantown, NY USA) were used in the developmental kindling studies. All animals were housed under standard environmental conditions with a constant ambient temperature of 23°C (60% humidity) and a 12:12 hr light:dark cycle with food and water available *ad-libitum*. On postnatal day 13 (P13, date of birth = P0) separate litters of animals were prepared for amygdala kindling. Under ketamine/xylazine anesthesia combination cannula/bipolar electrodes (25 gauge cannula equipped with stainless steel bipolar electrode, Plastics One Roanoke VA) were aimed at the left basolateral amygdala using stereotaxic techniques. The

coordinates for the basolateral amygdala for P13 rats were: 3mm posterior to bregma, 3.8mm lateral to the midline, and 8mm ventral to the skull surface with the incisor bar was set at -2.5mm. Upon completion of surgery animals were placed back with their dams and allowed to recover until P15 when the kindling procedure was started.

Kindling Retention—The kindling stimulation parameters consisted of biphasic square wave pulses of 1 sec duration with an amplitude of 400 μ A (peak to peak) delivered at 60 Hz [9]. Afterdischarges were amplified, digitized and recorded using a PC. In order to study the effect of PKM ζ inhibition on kindling retention we stimulated animals every 20 min until they achieved 5 consecutive stage 4 (bilateral forelimb clonus with rearing) or higher seizures [8]. The animals were then placed back with their dams for 24hrs before the rekindling was initiated. Twenty four hours after the final seizure animals were stimulated to ensure that they were kindled i.e. displayed stage 4 or higher seizures. Animals were then left to recover for 1 hour. Rats were then treated with either ZIP or a scrambled ZIP control peptide dissolved in PBS. ZIP (10 nmol) was infused into the basolateral amygdala in a volume of 1 μ l over 3 min; the control peptide was treated the same. This dose was selected based upon previous studies that found ZIP efficacious in the blockade of memory storage and LTP [19,21,24,25]. Thirty minutes later all animals were stimulated again and seizure severity (behavioral) and duration (EEG) was ascertained. In addition animals were also tested at 60 minutes post treatment and again at 24 hours to determine if there were any time related effects of ZIP.

Kindling Rate—To examine the effect of PKM ζ inhibition on kindling rate, a separate group of animals was used. In this experiment the stimulation parameters were the same but the kindling procedure was changed. On P15 animals were given a single AD producing stimulation. One hour later animals were treated with ZIP or the scrambled peptide and placed back in their home cages. Twenty four hours later animals restarted the kindling procedure and were followed to the same endpoint as in the previous experiment. Electrode placement was confirmed using posthoc histology.

Adult Rats

Animals and Surgery—Adult Sprague Dawley male rats (200 – 300g; approximately 1.5 – 3 months old) were equipped with combination cannula/bipolar electrode in the dorsal hippocampus (AP -3.8, ML -2.5, SI -2.8). After a 1.5 week recovery period, the animals underwent a rapid kindling protocol according to the procedures of Lothman & Williamson [14]. This began with an AD measurement (stimulus: 10s train 1ms biphasic pulses at 20 Hz, 0.5ms each phase), and continued with four alternate days of 12 stimulations each at a current suprathreshold to the initial threshold AD. Animals were considered kindled when they established and maintained Racine class 4 and 5 seizures in response to stimulations on the final day of kindling [20]. After establishment of the kindled state animals were left to rest for a period of 1 week. On the day of infusion, AD thresholds were again measured for each animal, subsequently each animal received 10 nmol of ZIP in 1 μ l PBS or scrambled ZIP slowly over 20 min while under light ketamine/xylazine anesthesia. On the day after infusion, AD thresholds were again measured in all animals. One hour later animals received 4 additional stimuli, separated by 1 hour, each at their respective kindling current. Behavioral and electrographic responses were ascertained throughout the kindling procedures. Electrode placement was also confirmed using posthoc histology.

Data Treatment and Statistical Analyses—Data are presented as means $\pm$ standard error of the mean (SEM). For the effects of ZIP on seizure severity and duration in developing animals, post treatment means were compared to baseline (in this case rekindled severity and duration) using paired t-tests. For the effects of ZIP on kindling rate means were compared

using a oneway ANOVA. For adult animals the severity of seizures after treatment was also compared to baseline using a paired t-test. Significance for all tests was set at $p < 0.05$.

RESULTS

Developing Rats

Kindling retention—After animals were fully kindled they were treated with ZIP or a scrambled control peptide and retested at 30 min, 60 min and 24 hr after treatment. Treatment with ZIP or the scrambled peptide did not affect kindling retention at any of the time points examined (figure 1A, B and C both groups $n = 4$) as all animals displayed similar seizure stages at all points tested. We also examined the effect of ZIP on AD duration recorded quantitatively on the EEG. Again we also did not find any significant changes in seizure duration due to ZIP at any time point tested, with only a minor trend towards a decrease in seizure duration at 24 hours (figure 1D, E, and F both groups $n = 4$).

Kindling rate—In a separate group of animals we examined the effect of ZIP on kindling rate. We found that ZIP given prior to kindling did not effect the rate at which developing animals kindled (figure 2A and B, naïve animals $n = 8$, scrambled ZIP $n = 5$ and ZIP $n = 5$).

Adult Rats—After rapid kindling, the AD thresholds of all animals were reduced. The day after ZIP injection, the AD thresholds of animals in both groups were slightly higher, with no difference seen between the experimental and control group (data not shown). All animals, regardless of injection status, responded to the stimuli with four Racine stage 4 or 5 generalized seizures, with the exception of one animal which had one stage 0 seizure followed by three generalized seizures. No obvious effect of ZIP was observed (figure 3, scrambled ZIP $n = 2$ and ZIP group $n = 3$).

DISCUSSION

In the current study we show that inhibition of PKM ζ does not affect kindling or kindling retention in either developing or adult rats. This was shown by the inability of the selective inhibitor ZIP to cause any significant alteration in kindling rate, kindling retention, and AD thresholds. This finding is in contrast to the significant role that has been established for PKM ζ in LTP and memory [21,24,25].

Both kindling and LTP are phenomena that can arise from high-frequency stimulation of discrete brain regions. At the core of each is a heavy reliance on activation of the glutamatergic system. Both rely on initial activation of NMDA receptors which results in increased intracellular Ca^{+2} that can influence a wide variety of intracellular signaling pathways [12, 16,18,28]. The lack of effect of PKM ζ in this study is not likely due to the dose of ZIP that we used because this dose has been shown to effectively block memory storage for a long periods of time and after strong training [19,25]. Thus we expected that if there were to be an effect on kindling that this dose would be sufficient. Since we did not even observe a trend towards any differences (other than seizure duration) it is unlikely that increasing the dose would have had much of an effect on the main variables of kindling retention and kindling rate.

This lack of effect is likely due to two factors. The first is the inability of ZIP to affect the local AD. The second factor is that kindling not only recruits networks outside the original focus by means of synaptic potentiation, but also significantly modifies them. In adult rats some of these modifications include synaptogenesis [13], neurogenesis [23], and neurodegeneration either in the focus [5], or in distal areas [10]. While it is true that *in vivo* LTP can produce synaptogenesis (mossy fiber sprouting in the dentate gyrus) it is not accompanied by other seizure/kindling induced alterations, such as cell loss and neurogenesis [1]. However, in the

absence of pathological changes (such as in developing rats [9]) ZIP also had no effect. This suggests that there may be other alterations involved in the recruitment and refinement of neural networks in kindling that are dissociated from PKM ζ activity and LTP.

Although this study is largely negative in nature, it is important to note that several compounds that affect LTP (for example NMDA antagonists) have shown efficacy in kindling. These may be important in the processes of the induction of epileptogenesis (without SE and spontaneous seizures) and are worth examining in the hunt for new antiepileptic therapies. Our results indicate, however, that the maintenance mechanisms that sustain epileptogenesis and behavioral memory are distinct. This gives hope that the molecular mechanisms that sustain pathophysiological hyperexcitability may be ameliorated without affecting the storage mechanism of long-term memories.

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References

1. Adams B, Sazgar M, Osehobo P, Van der Zee CE, Diamond J, Fahnestock M, Racine RJ. Nerve growth factor accelerates seizure development, enhances mossy fiber sprouting, and attenuates seizure-induced decreases in neuronal density in the kindling model of epilepsy. *J Neurosci* 1997;17:5288–5296. [PubMed: 9204913]
2. Albensi BC, Oliver DR, Toupin J, Odero G. Electrical stimulation protocols for hippocampal synaptic plasticity and neuronal hyper-excitability: are they effective or relevant? *Exp Neurol* 2007;204:1–13. [PubMed: 17258711]
3. Bliss TVP, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 1973;232:331–356. [PubMed: 4727084]
4. Blumenfeld H, Rivera M, Vasquez JG, Shah A, Ismail D, Enev M, Zaveri HP. Neocortical and thalamic spread of amygdala kindled seizures. *Epilepsia* 2007;48:254–262. [PubMed: 17295618]
5. Bolkvadze TA, Dzhaparidze ND, Zhvaniya MG, Bikashvili TZ, Chilachava LR. Kindling-induced hippocampal cell death in rats. *Neurosci Behav Physiol* 2008;38:359–362. [PubMed: 18401726]
6. Drier EA, Tello MK, Cowan M, Wu P, Blace N, Sacktor TC, Yin JC. Memory enhancement and formation by atypical PKM activity in *Drosophila melanogaster*. *Nat Neurosci* 2002;5:316–324. [PubMed: 11914720]
7. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969;25:295–330. [PubMed: 4981856]
8. Haas K, Sperber EF, Moshé SL. Kindling in developing animals: expression of severe seizures and enhanced development of bilateral foci. *Dev Brain Res* 1990;56:275–280. [PubMed: 2261687]
9. Haas KZ, Sperber EF, Opanashuk LA, Stanton PK, Moshe SL. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus* 2001;11:615–625. [PubMed: 11811655]
10. Heida JG, Teskey GC, Pittman QJ. Febrile convulsions induced by the combination of lipopolysaccharide and low-dose kainic acid enhance seizure susceptibility, not epileptogenesis, in rats. *Epilepsia* 2005;46:1898–1905. [PubMed: 16393155]
11. Holmes GL. Animal model studies application to human patients. *Neurology* 2007;69:S28–32. [PubMed: 18071155]
12. Lamsa K, Irvine EE, Giese KP, Kullmann DM. NMDA receptor-dependent long-term potentiation in mouse hippocampal interneurons shows a unique dependence on Ca(2+)/calmodulin-dependent kinases. *J Physiol* 2007;584:885–894. [PubMed: 17884930]

13. Li S, Reinprecht I, Fahnstock M, Racine RJ. Activity-dependent changes in synaptophysin immunoreactivity in hippocampus, piriform cortex, and entorhinal cortex of the rat. *Neuroscience* 2002;115:1221–1229. [PubMed: 12453493]
14. Lothman EW, Williamson JM. Rapid kindling with recurrent hippocampal seizures. *Epilepsy Res* 1993;14:209–220. [PubMed: 8504791]
15. McEachern JC, Shaw CA. The plasticity-pathology continuum: defining a role for the LTP phenomenon. *J Neurosci Res* 1999;58:42–61. [PubMed: 10491571]
16. Morimoto K, Fahnstock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol* 2004;73:1–60. [PubMed: 15193778]
17. Moshé SL. The effects of age on the kindling phenomenon. *Dev Psychobiol* 1981;14:75–81. [PubMed: 7274579]
18. Nicoll RA, Malenka RC. Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. *Ann N Y Acad Sci* 1999;868:515–525. [PubMed: 10414328]
19. Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. *Science* 2006;313:1141–1144. [PubMed: 16931766]
20. Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizures. *Electroencephal Clin Neurophysiol* 1972;32:281–294.
21. Sacktor TC. PKMzeta, LTP maintenance, and the dynamic molecular biology of memory storage. *Prog Brain Res* 2008;169:27–40. [PubMed: 18394466]
22. Schubert M, Siegmund H, Pape HC, Albrecht D. Kindling-induced changes in plasticity of the rat amygdala and hippocampus. *Learn Mem* 2005;12:520–526. [PubMed: 16204204]
23. Scott BW, Wang S, Burnham WM, De Boni U, Wojtowicz JM. Kindling-induced neurogenesis in the dentate gyrus of the rat. *Neurosci Lett* 1998;248:73–76. [PubMed: 9654345]
24. Serrano P, Yao Y, Sacktor TC. Persistent phosphorylation by protein kinase Mzeta maintains late-phase long-term potentiation. *J Neurosci* 2005;25:1979–1984. [PubMed: 15728837]
25. Shema R, Sacktor TC, Dudai Y. Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. *Science* 2007;317:951–953. [PubMed: 17702943]
26. Soderling TR, Derkach VA. Postsynaptic protein phosphorylation and LTP. *Trends Neurosci* 2000;23:75–80. [PubMed: 10652548]
27. Teyler TJ, DiScenna P. The role of hippocampus in memory: a hypothesis. *Neurosci Biobehav Rev* 1985;9:377–389. [PubMed: 2999655]
28. Yamagata Y, Obata K. Ca²⁺/calmodulin-dependent protein kinase II in septally kindled rat brains: changes in protein level, activity and subcellular distribution in hippocampus and cerebral cortex. *Neurosci Lett* 1996;211:109–112. [PubMed: 8830856]

FIGURE 1.

The effect of ZIP and control scrambled peptide on amygdala kindling seizure severity (retention) and duration in developing rats. Seizure severity is shown in the top panels (A, B, and C). There were no differences between baseline and either treatment at any time point measured ($p > 0.6$). The effect of ZIP and scrambled peptide on seizure duration is shown in the lower panels (D, E, and F) and again there is no effect at any time point tested ($p > 0.1$) both groups $n = 4$.

FIGURE 2.

The effect of ZIP and control scrambled peptide on kindling rate in developing rats. Rate of kindling to full seizure generalization (A) was not affected by ZIP ($p > 0.6$). Kindling through the initial phase (partial seizures) was also unaltered (B, $p > 0.6$), naïve group $n = 8$, control (scrambled ZIP) $n = 5$ and ZIP $n = 5$.

FIGURE 3.

The effect of ZIP and control scrambled peptide on kindling seizure severity (retention) in adult rats. Similar to developing rats there were no effect of ZIP or scrambled peptide on kindling retention ($p > 0.7$) control (scrambled ZIP) $n = 2$, and ZIP group $n = 3$.