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Evaluation of development-specific targets for antiepileptogenic therapy using rapid kindling

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Abstract

We used the method of rapid hippocampal kindling to assess the potential antiepileptogenic efficacy of a number of anticonvulsant medications. This method afforded a higher throughput than methods based on traditional kindling or post-status epilepticus models of epileptogenesis. This “compressed epileptogenesis” model also permitted the study of age-dependent pharmacologic targets, and distinguished among AEDs based on their age-specific antiepileptogenic efficacy. We found retigabine to be the most effective anticonvulsant therapy during early development. Topiramate seemed most effective further along development, while some drugs did not demonstrate an age-specific effect. The method also reproduced some of the paradoxical pharmacologic findings previously shown with lamotrigine. While the utility of this model for screening the antiepileptogenic therapies requires further validation it introduces the ability to undertake development-specific testing and a more rapid throughput than conventional methods.

Keywords

Epileptogenesis; development; rapid kindling; hippocampus

In March of 2007, researchers, physicians, patients, family members and voluntary health organization leaders came together on the NIH campus to participate in a conference called “Curing Epilepsy 2007: Translating Discoveries into Therapies,” which established a set of epilepsy research benchmarks. Benchmark area I (Prevent epilepsy and its progression) included sub-goal E, stated as “develop new animal models to study epileptogenesis.” In addition, benchmark area II (Develop new therapeutic strategies and optimize current approaches to cure epilepsy), in one of the recommendations under sub-goal C states

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"develop higher-throughput cost-effective models for screening pharmacotherapies for specific types of epilepsy."

Our laboratory has been interested in approaches to meeting the challenges relevant to the practical development of therapies that may prevent the development of epilepsy or at least, retard the progression of the epileptogenic process. The preclinical approaches and challenges in developing therapy to modify the course of epilepsy have been reviewed (Stables et al., 2003; White, 2003). Traditional and established approaches to studying epileptogenesis such as the kindling or the post-status epilepticus models (Morimoto et al., 2004) involve sufficient duration of observation as to be not able to meet a benchmark for high throughput. High throughput is essential for the screening of large number of compounds in order to get an initial signal regarding the suitability for moving a test compound further along the road to translation for clinical trials.

We are also particularly interested in the developmental aspects of epileptogenesis and the ontogeny of distinct mechanisms that may represent a spectrum of targets for antiepileptogenic interventions. In studying epileptogenesis during development, method development must acknowledge the problems posed by the rapid growth of the immature brain and skull such that carefully placed electrodes for procedures such as kindling may be displaced. It became apparent that a process that represents "compressed epileptogenesis" may bring feasibility to being able to study the process during distinct developmental stages as well as improve throughput. We proposed that a rapid kindling model, initially described by Lothman and coworkers (Lothman et al., 1985; Michelson and Lothman, 1991) and involving the stimulation of the ventral hippocampus might permit the study of the effect of an AED on disease progression much more rapidly than the traditional kindling model or post-status epilepticus epilepsy.

We have studied the effect of a number of pharmacologically distinct treatments in rat pups ranging in age from postnatal day 11 (P11, neonatal) to P35 (adolescent) on baseline hippocampal excitability as measured by after discharge thresholds (ADT) and durations (ADD), kindling acquisition (number of stimulations to stage 1 and 4 seizures, number and duration of stage 4 seizures). Determination of ADT and ADD involved stimuli of 10 s duration, 20 Hz, 1 ms pulse duration in square wave monophasic pulses. The kindling procedure involved 60 trains delivered every 5 min using a current 100 μA over the ADT (total procedure duration 5 h). Behavioral seizures were scored using the following scale: 1—Motor arrest and twitching vibrissae; 2—chewing, head bobbing; 3—forelimb clonus; 4—forelimb clonus and rearing; 5—rearing and falling. In addition, the afterdischarge properties were determined again 24 h after the kindling experiments to assess the retention of kindling. We also tested the ability of the test treatment to block evoked seizures produced by a threshold stimulation, 24 h after rapid kindling.

The rapid kindling paradigm has not been reported to produce a lasting or stable kindled state. We have seen retention of kindling in developing animals 2 and 4 weeks after kindling, but less reliably so at 10 weeks. Our aim was to assess the effect of an AED on the rate of acquisition of kindling, rather than to produce permanent epilepsy. We focused on the "journey" representing the epileptogenic process, rather than the "destination" of lasting spontaneous recurrent seizures.

Our experience with a mechanistic study of galanin receptor subtypes using rapid kindling (Mazarati et al., 2006) in mature rats had revealed that the method yielded results comparable to those in experiments using traditional kindling with once daily stimulations in galanin overexpressing rats (Kokaia et al., 2001). Topiramate (TPM) is an anticonvulsant with many known mechanisms of action, but its ability to block AMPA-mediated currents

have been considered to be more consistent than its effects on voltage-gated sodium channels and GABA receptors. In our rapid-kindling experiments, TPM treatment demonstrated little effect on baseline ADT or ADD in P14 and P21 pups, but elevated the ADT and shortened the ADD in the P35 animals (Mazarati et al., 2007). The number of stimulations required to elicit a stage 1 or sage 4 seizures was increased in P21 and P35 rats, and these findings were reminiscent of the results obtained in mature rats using traditional amygdaloid kindling (Amano et al., 1998). Interestingly, TPM did not retard kindling acquisition in P14 rats, demonstrating a development-specific effect on this phenomenon. Thus, TPM was least effective in modifying kindling acquisition in the youngest age (P14) tested, which contrasts with the impressive antiepileptogenic effect it demonstrated in the lithium-pilocarpine induced post-status epilepticus model (Suchomelova et al., 2006); in those experiments, treatment with TPM was more effective in P15 animals compared to P28 rats. The antiepileptogenic effect was discernible despite its modest effect on seizures comprising the status epilepticus episode. Acute treatment with TPM was effective in blocking evoked seizures in kindled animals of all ages tested, demonstrating that it is an anticonvulsant at P14, but not antiepileptogenic. In the P35 animals TPM displayed anticonvulsant effects as well as an ability to retard the process of epileptogenesis.

We studied retigabine (RTG), and investigational AED, which is known to augment the so-called M-type K^+ currents by promoting the opening of KCNQ2/3 channels (Kv7.2/7.3). Deficiency of these currents are known to selectively enhance the excitability of the brain in the very immature brain, when GABA-induced currents are still depolarizing rather than hyperpolarizing. This is believed to form the basis of benign familial neonatal conventions. Thus we expected that RTG may be especially effective in antagonizing kindling development in the very young animals. We found that RTG treatment had a significant effect on baseline afterdischarge properties (ADT and ADD) in P14, P21, and P35 animals (Mazarati et al., 2008). Moreover, RTG interfered with kindling development in rats at each stage of development tested. Post-acquisition experiments designed to examine kindling retention showed that the P35 rats did show enhanced excitability 24 h after kindling, but this was not the case with P14 or P21 animals, demonstrating a greater efficacy of RTG in the younger animals (P14 and P21 compared to P35). Thus, the developmental profile of the antiepileptogenic efficacy of TPM and RTG are quite distinct.

Immature neurons display a paradoxical depolarizing response to GABAergic stimulation, (Ben-Ari, 2002) which has been attributed to the high expression of the chloride accumulating neonatal Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), and the low expression of the chloride extruding K^+-Cl^- cotransporter (KCC2) (Yamada et al., 2004). Consistent with those findings, acute anticonvulsant effects in the neonatal brain could be demonstrated in response to treatment with bumetanide (BUM) (Dzhala et al., 2005), a loop diuretic and selective blocker of KCC1 (Hannaert et al., 2002). We investigated the antiepileptogenic potential of BUM in our rapid kindling model (Mazarati et al., 2009). The baseline afterdischarge properties in P14 and P21 rats were not affected by treatment with BUM. In P11 animals, a modest effect on ADT (elevated) and ADD (lowered) could be demonstrated. Kindling acquisition was retarded only in P11 rat pups. Thus, the results obtained with rapid kindling are consistent with the known ontogeny of chloride transport during development.

We have less complete data on other treatments which have not been published elsewhere, but are summarized in Table 1. Experiments with ganaxalone showed a paradoxical effect on stage 1 seizures (increased number of seizures, and seizure duration) which may reflect the preferential effect of this drug on extrasynaptic GABA receptors mediating tonic inhibition. There was no effect on the baseline afterdischarge properties in the youngest rat pups (P14, P21) tested. Treatment with lamotrigine (LTG) delayed kindling acquisition as shown in traditional kindling experiments with mature rats (Stratton et al., 2003). However,

LTG was not effective in treating kindled seizures in the rats exposed to LTG during kindling, reminiscent of the observation by Postma et al. (2000). Thus, results with rapid kindling in immature rats reflect some of the pharmacologic findings noted in the published literature using traditional kindling in mature animals. No effect of LTG treatment was seen in a post-status epilepticus model of epileptogenesis (Nissinen et al., 2004). Experiments with levetiracetam produced results concordant with data from adult animals in a traditional kindling study (Löscher et al., 1998) – however, no developmental effect was discernible in our studies at P14, P21 and P35.

We also undertook a very limited set of experiments (limited by availability of the test compound) by subjecting P35 rats to rapid kindling, using a rapamycin analog. This compound is not an AED and is known for its effect on inhibiting the mTOR (mammalian target of rapamycin) pathway. This pathway has been implicated in the epileptogenic process in tuberous sclerosis and rapamycin affected epileptogenesis in a mouse model of that disease (Zeng et al., 2008). Recently, it was also shown to have an antiepileptogenic effect in a post-status epilepticus model of epileptogenesis employing kainic acid (Zeng et al., 2009). In our experiments, four days of treatment had no effect on baseline afterdischarge properties or the rate of kindling acquisition. However, in the post-acquisition (24 hr) test, threshold stimulation failed to evoke a seizure in rats kindled in the presence of the rapamycin analog. Thus, rapid kindling methods may serve the need for screening antiepileptogenic therapies that may not acutely impact on brain excitability with good throughput.

The utility of rapid kindling as a model of “compressed” epileptogenesis remains to be validated more completely. It is not advanced as a replacement for studies involving epileptogenesis that follows status epilepticus. Our purpose has been to explore a method in which candidate antiepileptogenic therapies can be compared for antiepileptogenic activity with a more rapid throughput than is possible with traditional kindling, and also accommodate young animals during a stage of rapid brain growth. Rapid kindling is likely better suited to studying the early molecular and cellular changes than the long-term circuit rearrangements seen in chronic TLE. It remains to be demonstrated if intervention with the early molecular and cellular processes can prove a useful strategy to minimize the long-term consequences.

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References

1. 2007 NINDS Epilepsy Research Benchmarks. http://www.ninds.nih.gov/research/epilepsyweb/2007_benchmarks.htm#benchmarks
2. Amano K, Hamada K, Yagi K, Seino M. Antiepileptic effects of topiramate on amygdaloid kindling in rats. Epilepsy Research 1998;31:123–128. [PubMed: 9714503]
3. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. Nat Rev Neurosci 2002;3:728–739. [PubMed: 12209121]
4. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. Nat Med 2005;11:1205–1213. [PubMed: 16227993]
5. Kokaia M, Holmberg K, Nanobashvili A, Xu ZQ, Kokaia Z, Lendahl U, Hilke S, Theodorsson E, Kahl U, Bartfai T, Lindvall O, Hökfelt T. Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. Proc Natl Acad Sci U S A 2001;98:14006–14011. [PubMed: 11698649]

6. Löscher W, Hönack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J Pharmacol Exp Ther 1998;284:474–479. [PubMed: 9454787]
7. Lothman EW, Hatlelid JM, Zorumski CF, Conry JA, Moon PF, Perlin JB. Kindling with rapidly recurring hippocampal seizures. Brain Res 1985;360:83–91. [PubMed: 3000538]
8. Mazarati A, Lundström L, Sollenberg U, Shin D, Langel U, Sankar R. Regulation of kindling epileptogenesis by hippocampal galanin type 1 and type 2 receptors: The effects of subtype-selective agonists and the role of G-protein-mediated signaling. J Pharmacol Exp Ther 2006;318(2): 700–708. [PubMed: 16699066]
9. Mazarati A, Shin D, Auvin S, Sankar R. Age-dependent effects of topiramate on the acquisition and the retention of rapid kindling. Epilepsia 2007;48:765–773. [PubMed: 17319916]
10. Mazarati A, Wu J, Shin D, Kwon YS, Sankar R. Antiepileptogenic and antiictogenic effects of retigabine under conditions of rapid kindling: An ontogenic study. Epilepsia 2008;49:1777–1786. [PubMed: 18503560]
11. Mazarati A, Shin D, Sankar R. Bumetanide inhibits rapid kindling in neonatal rats. Epilepsia 2009;50:2117–22. [PubMed: 19260939]
12. Michelson HB, Lothman EW. An ontogenetic study of kindling using rapidly recurring hippocampal seizures. Brain Res Dev Brain Res 1991;61:79–85.
13. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. Progress in Neurobiology 2004;73:1–60. [PubMed: 15193778]
14. Nissinen J, Large CH, Stratton SC, Pitkänen A. Effect of lamotrigine treatment on epileptogenesis: an experimental study in rat. Epilepsy Res 2004;58:119–132. [PubMed: 15120743]
15. Postma T, Krupp E, Li XL, Post RM, Weiss SR. Lamotrigine treatment during amygdala-kindled seizure development fails to inhibit seizures and diminishes subsequent anticonvulsant efficacy. Epilepsia 2000;41:1514–1521. [PubMed: 11114208]
16. Stables JP, Bertram E, Dudek FE, Holmes G, Mathern G, Pitkänen A, White HS. Therapy discovery for pharmacoresistant epilepsy and for disease-modifying therapeutics: summary of the NIH/NINDS/AES models II workshop. Epilepsia 2003;44:1472–1478. [PubMed: 14636315]
17. Stratton SC, Large CH, Cox B, Davies G, Hagan RM. Effects of lamotrigine and levetiracetam on seizure development in a rat amygdala kindling model. Epilepsy Res 2003;53:95–106. [PubMed: 12576171]
18. Suchomelova L, Baldwin RA, Kubova H, Thompson KW, Sankar R, Wasterlain CG. Treatment of experimental status epilepticus in immature rats: dissociation between anticonvulsant and antiepileptogenic effects. Pediatr Res 2006;59:237–243. [PubMed: 16439585]
19. White HS. Preclinical development of antiepileptic drugs: past, present, and future directions. Epilepsia 2003;44(suppl. 7):2–8. [PubMed: 12919332]
20. Yamada J, Okabe A, Toyoda H, Kilb W, Luhmann HJ, Fukuda A. Cl- uptake promoting depolarizing GABA actions in immature rat neocortical neurons is mediated by NKCC1. J Physiol 2004;557:829–841. [PubMed: 15090604]
21. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. Ann Neurol 2008;63:444–453. [PubMed: 18389497]
22. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci 2009;29:6964–6972. [PubMed: 19474323]

Table 1

Effect of treatments on measures of hippocampal excitability in rats of different developmental stages that underwent rapid kindling

Test Compound	Baseline hippocampal excitability	Kindling acquisition: stage 1 seizures	Kindling acquisition: Total number of stage 4 seizures	Effect on Kindling retention	Effects on kindled seizures
Topiramate	Decreased	Reduced	Reduced	No	Yes
Retigabine	Yes, P14, 21, 35	Only reduced in P14, not P21 and 35	Blocked at all ages	Yes at P14 & 21; No at P35	Effective at all ages
Bumetanide	No effect in 21, worked at all paramaters at P11, and somewhat at P14				
Ganaxolone, 40 mg/kg	Not changed P35 rats only	Number of seizures increased; seizure duration increased	Number of SZ decreased; SZ duration not changed	Yes	Not studied
Lamotrigine, 30 mg/kg	Decreased, P35 rats only	Delayed	Delayed/reduced	No	No
Rapamycin analog, 10 mg/kg q 12 h × 4d	Not changed, P 35 rats only	Not changed	Not changed	AD-not changed; SZ at AD attenuated	
Levetiracetam, 60 mg/kg	Decreased	No effect	Delayed/reduced	Yes	Yes