Kindling Induces Long-Lasting Alterations in Response of Hippocampal Neurons to Elevated Potassium Levels in vitro

Abstract. *The cellular basis of kindling was studied electrophysiologically with slices of guinea pig hippocampus. Normally, epileptiform activity can be induced in the slices only by combined exposure to elevated potassium levels and a chemical convulsant such as penicillin. In hippocampal slices from pentylenetetrazole-kindled animals, however, elevated potassium alone can induce seizures. These data suggest that kindling elicits long-term changes in neuronal excitability that may involve ionic mechanisms.*

Kindling, the progressive lowering of seizure thresholds with repeated stimulation, has been demonstrated in several different brain areas, most notably the hippocampus and other limbic regions. Hippocampal kindling, induced with electrical (*1*) or chemical (*2*) methods, leads to prolonged, perhaps irreversible changes in neuronal excitability that are often studied as a model for epilepsy. Kindling has been chemically induced by daily administration of convulsant or subconvulsant doses of several excitatory agents including acetylcholine, pentylenetetrazole (PTZ), and lidocaine (*3*-*5*). Although there is evidence for the involvement of cholinergic (*6*) and catecholaminergic (*7*) systems in kindling, little is known of the physiological substrate. In part, this uncertainty reflects difficulties in studying epileptiform discharge in the living, intact animal, in which seizures are often accompanied by marked motor changes and hypoxia.

Recently, we reported on conditions necessary for generating epileptiform activity in hippocampal slices in vitro (*8*). In this preparation, seizure activity was readily maintained without the constitutional or indirect changes seen in the intact animal. We found that exposure to both elevated K^+ and a chemical convulsant such as penicillin is necessary to elicit seizures in hippocampal slices from normal animals. Once triggered, the epileptiform activity can be modulated by alterations in Ca^{2+} concentrations, with decreased levels increasing the frequency of interictal spikes (IIS's) and increased levels decreasing that frequency (*9*). (Calcium concentration changes alone, however, cannot initiate epileptiform activity.) Finally, the rate of IIS discharge is consistently related to the steady-state concentration of K^+ in the medium (*8*).

Since we could define many of the initiating and modulatory influences for epileptiform activity in an isolated mammalian central nervous system preparation, we felt that the hippocampal slice would be ideal for studying any changes elicited by kindling. We now report that slices from kindled animals do not require a chemical convulsant, but manifest epileptiform activity with elevated K^+ alone. Furthermore, this change persists for at least 20 days after the last kindling trial.

Groups of male Hartley guinea pigs (seven per group) were kindled with PTZ (50 mg/kg per day, intraperitoneally) for 1 to 5 days (*10*), and drug-induced seizures were evaluated behaviorally (*11*). The animals were killed 3 to 20 days after the last injection, and slices of hippocampus were prepared (*12*).

Little change in spontaneous action potential activity of single cells was seen in the slices from kindled animals. However, sustained spontaneous IIS discharges (*9*) were triggered in kindled animals simply by elevating K^+ levels (Fig. 1A), without addition of a chemical convulsant. Interestingly, the rate of IIS discharge in slices from kindled animals with various levels of elevated extracellular K^+ was strikingly similar to IIS discharge rates in slices from normal animals exposed to penicillin and similar levels of K^+ (*12*). In many other respects there was little difference in comparison with slices from normal animals. Thus tetanizing electrical stimuli did not trigger sustained IIS's (Fig. 1C). Interictal spike frequency was increased by reducing extracellular calcium concentration and decreased by increasing it (Fig. 1B).

Several considerations suggest that the alteration in the electrophysiological properties of the slice are causally related to the preceding period of kindling. First, the probability of abnormal epileptiform discharge triggered by elevated K^+ alone was clearly related to the number of seizures (and PTZ injections) given during the kindling period (Fig. 2A). Slices from fully kindled animals had a

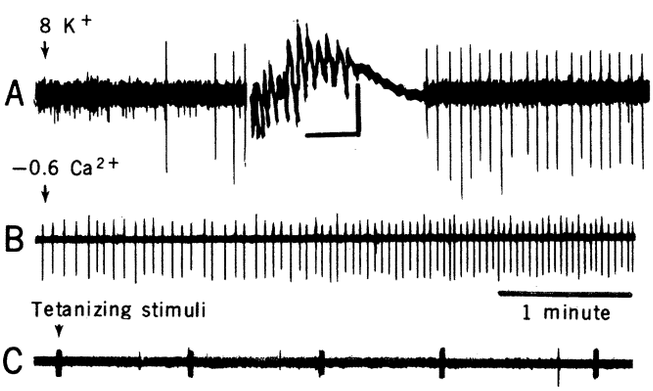

Fig. 1. Characteristics of IIS's in kindled slices. (A) Interictal spikes initiated in kindled slices by increasing the concentration of K^+ (8K = 8 m*M* K^+). Spiking is rhythmic and of large amplitude. Above the calibration marks is an oscilloscope trace made at high sweep speed showing the detailed configuration of an IIS as described in (*9*) (horizontal calibration, 20 msec; vertical calibration, 500 μV). (B) Reduction of Ca^{2+} concentration by 0.6 m*M* increases the frequency of spiking in medium containing 8 m*M* K^+. The normal Ca^{2+} concentration in superfusion medium is 2.4 m*M*. (C) Tetanizing stimuli (arrow) do not cause rhythmic discharge at the normal concentration of K^+ (6.2 m*M*). Tetanizing stimuli were delivered to the fimbria via a concentric electrode. Stimulus parameters were as described in (*12*).

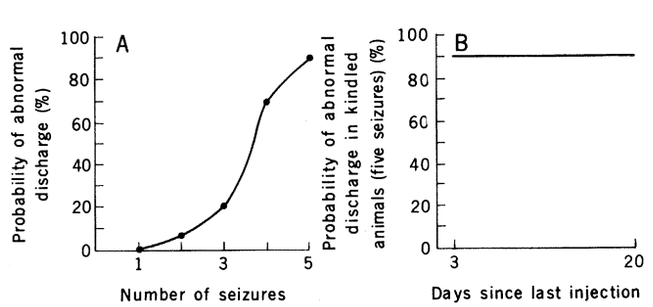

Fig. 2. (A) The probability of obtaining spontaneous IIS's with elevated K^+ concentrations is dependent on the number of PTZ-induced seizures. Each point on the curve is derived from data for at least two slices from each of seven animals. (B) Probability of spontaneous abnormal discharge induced by K^+ remains high for at least 20 days after the last injection.

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virtually 100 percent probability of manifesting IIS's when challenged with elevated K^+ alone; fewer PTZ injections reduced this probability in a dose-dependent manner (Fig. 2A). Second, in animals given five PTZ injections, the K^+-induced epileptiform discharge persisted for at least 20 days (Fig. 2B) after the last injection (*13*). In rodents, less than 5 percent of an injected dose of PTZ remains in the brain after 24 hours (*14*). Furthermore, no spontaneous convulsions were observed after termination of the injections, suggesting that there was not a prolonged drug exposure at convulsant levels. However, this is, at best, indirect evidence that does not entirely preclude the presence of some small level of compartmentalized PTZ in the tissue. To test this possibility, we placed enough PTZ (5 to 10 μg/ml) in the superfusion medium of control slices to generate IIS directly, and recorded the washout time. Enough PTZ could be washed out of the slice in 1 hour to terminate spontaneous spiking. Together with the previously cited data, this result makes it seem unlikely that residual PTZ is responsible for the heightened excitability found in kindled slices.

In conclusion, our data show that hippocampal slices from kindled animals no longer require a chemical convulsant in the medium to trigger epileptiform activity when the concentration of K^+ is elevated. Since the sensitivity to elevated K^+, modulatory influences of divalent cations, and responses to electrical tetanization are similar to those seen in slices from control animals, we hypothesize that kindling induces a change in basic membrane or synaptic properties similar to that induced acutely by convulsants such as penicillin. In view of the postulated relation between kindling and epilepsy (*15*) and enduring neuropsychological changes such as learning and memory (*16*), future research employing the model described in this report may provide important insights into normal and abnormal neuronal function.

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9. The IIS's recorded here, consisting of biphasic bioelectric potentials of large amplitude and long duration, are extracellular manifestations of paroxysmal depolarizing shifts. This correlation was well established in epilepsy models in vivo [D. A. Prince, *Exp. Neurol.* **21**, 307 (1968); M. A. Dichter and W. A. J. Spencer, *Neurophysiology (USSR)* **32**, 663 (1969)], and in hippocampal slices in vitro [A. P. Oliver, B. J. Hoffer, R. J. Wyatt, *Epilepsia* **18**, 543 (1977); A. P. Oliver *et al.* (*8*); A. P. Oliver, J. S. Carman, B. J. Hoffer, R. J. Wyatt, *Exp. Neurol.*, in press].
10. The kindling procedure with PTZ is similar to that used in other laboratories [T. Ito, M. Hori, K. Yoshida, M. Shimizu, *Eur. J. Pharmacol.* **45**, 165 (1977); S. T. Mason and M. E. Corcoran, *Brain Res.* **170**, 497 (1979)].
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12. Each slice was about 350 μm thick. Details of our techniques have been described (*9*). The basic medium for superfusion had the following composition (millimoles per liter): NaCl, 124; KCl, 5; $CaCl_2$, 2.4; $MgSO_4$, 1.3; KH_2PO_4, 1.24; glucose, 10; and $NaHCO_3$, 26. Sodium penicillin G (Sigma) or concentrated K^+ or both were added to the basic perfusion medium in some experiments. All recordings were obtained from the CA_3 region of the slice with a glass micropipette filled with 3*M* NaCl (impedance, 1 to 5 megohms), amplified by conventional means, and displayed on an oscilloscope and a strip-chart recorder. The IIS frequency was counted directly from the strip-chart record. Electrical stimulation of the fimbria was performed with 10 to 20 repetitive stimuli per second. Each stimulus was a 10-V monophasic square wave lasting 50 μsec.
13. An obvious question in the kindling paradigm concerns possible disruption of the hippocampal inhibitory interneurons secondary to post-seizure hypoxia. Selective vulnerability of inhibitory interneurons to hypoxia has been reported for the spinal cord [R. A. Davidoff, L. T. Graham, R. P. Shank, R. Werman, M. Aprison, *J. Neurochem.* **14**, 1025 (1967)] and the hippocampus [T. Dunwiddie, A. Mueller, M. Palmer, J. Stewart, B. Hoffer, *Brain Res.*, in press]. Several observations suggest that this problem did not occur in our experiments. First, most animals studied physiologically manifested hyperpnea during and after PTZ-induced seizures, an effect well documented in the literature. Second, no spontaneous seizures were observed between the last kindling trial and physiological testing of the slices. Finally, action potential discharge in single pyramidal neurons in kindled slices, prior to elevating the K^+ concentration, resembled that seen in normal slices, again suggesting intact inhibitory mechanisms.
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17. We thank T. Dunwiddie for critical evaluation of the manuscript.

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Cloned Cauliflower Mosaic Virus DNA Infects Turnips (*Brassica rapa*)

Abstract. *Cauliflower mosaic virus DNA cloned in the Sal I site of bacterial plasmid pBR322 infects turnip plants. The cloned viral DNA must be excised from the recombinant plasmid to infect, but need not be circularized and ligated in vitro. The cloned viral DNA lacks site-specific single-strand breaks found in DNA obtained directly from the virus. However, these breaks are reintroduced into the viral genome during multiplication of the virus in the plant host.*

Cauliflower mosaic virus (CaMV) has attracted attention in the past few years because of its possible use in plant "genetic engineering" studies. Cauliflower mosaic virus is one of the few plant viruses containing double-strand DNA, and the DNA, when isolated from the virus, can infect plants (*1*). These properties have led to the suggestion that the CaMV DNA could be used as a vehicle for introducing foreign DNA in plant cells (*2*) in much the same fashion as SV40 viral DNA has been used to introduce DNA in animal cells (*3*). Since the construction of a CaMV DNA vehicle would involve recombinant DNA technology, it is important to show first that cloned CaMV DNA can infect plants.

Szeto *et al.* (*2*) dampened much of the optimism for using CaMV DNA as a vehicle in plants. They found that plants could not be infected by cloned CaMV DNA or by CaMV DNA cleaved at a single site by a restriction enzyme and religated in vitro. They attributed the failure to one of several factors, including the possibility that some secondary structure of the DNA, crucial for infectivity, was lost when the viral DNA was cleaved (*2*).

Since the report by Szeto *et al.* (*2*), several studies have shown that, indeed, the CaMV genome has an unusual secondary structure. The genome isolated from the virus is found in both linear and open (noncovalently closed) circular forms (*4*) with two or three (depending

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