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Laboratory Research

Calorie Restriction and Ketogenic Diet Diminish Neuronal Excitability in Rat Dentate Gyrus In Vivo

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Summary: *Purpose:* The ketogenic diet (KD) is an effective treatment for intractable epilepsy. However, little is known about its underlying mechanisms.

Methods: In this study, in vivo extracellular field responses to angular bundle stimulation were recorded in the dentate gyrus of Sprague–Dawley rats fed one of three diets: ketogenic calorie-restricted (KCR), normal calorie-restricted (NCR), or normal ad libitum (NAL). Input/output curves and paired-pulse relations were used to assess network excitability. A maximal dentate activation (MDA) protocol was used to measure electrographic seizure threshold and duration.

Results: Animals fed calorie-restricted (CR) diets exhibited greater paired-pulse inhibition, an elevated MDA threshold, and an absence of spreading depression-like events compared with ad libitum-fed controls. In the MDA model of epileptogenesis, the rate of increase in electrographic seizure duration after repeated stimuli was markedly reduced in KCR-fed animals compared with NCR- and NAL-fed controls.

Conclusions: These data suggest that CR, by itself, can be anticonvulsant, and treatment with a KCR diet may be both anticonvulsant and antiepileptogenic. **Key Words:** Ketogenic diet—Calorie restriction—Epilepsy—Dentate gyrus—β-Hydroxybutyrate.

Epilepsy affects ~50 million people worldwide (1) and is defined symptomatically by the appearance of recurrent spontaneous seizures. Pharmacologic treatments to date, however, have been aimed chiefly at treating the symptom of epilepsy—seizures—rather than its cause. Because ~20% of patients are resistant to current anticonvulsant drugs (AEDs), the elucidation of treatments that can inhibit the development of epilepsy would represent an essential first step in preventing or curing the family of seizure disorders.

The ketogenic diet (KD) is a long-standing treatment for intractable pediatric epilepsy. Numerous clinical reports have shown that approximately half of all patients maintained on the KD exhibit ≥50% reduction in seizures (2), irrespective of patient gender or of seizure type (3,4). The diet was originally formulated 80 years ago to mimic the biochemical changes observed during fasting (5). In fasting, when glucose sources are limited, ketone bodies are produced in the liver and used by the brain as an alternative metabolic substrate (6). As ketonemia has been positively correlated with improved seizure control (7), the goal of KD treatment has been to produce and maintain ketonemia. Toward this end, the KD regimen also is often calorie restricted to 10–25% of daily recommended calories; clinical reports ascribe the most common error in initiating and maintaining the KD to overestimation of caloric requirements and resultant weight gain (3,8).

Animal studies have confirmed an anticonvulsant role for the KD in both mice (9,10) and rats (11,12). However, it is still not clear how the diet works or whether it can prevent the development of epilepsy. Few experimental studies have explored the anticonvulsant/antiepileptic mechanisms of the KD, perhaps because of difficulties in replicating the relevant biochemical milieu in vitro (i.e., standard culture-bathing medium contains glucose that is likely to counter the "ketotic" environment). We therefore approached our analysis of the KD by carrying out in vivo extracellular field recordings in the dentate gyrus of animals fed one of three diets: ketogenic calorie-restricted (KCR), normal calorie-restricted (NCR), or normal ad libitum (NAL). The dentate was chosen because of its important role in modulating hippocampal excitability (13) and its relevance to processes of epileptogenesis (14). Input/output and paired-pulse electrophysiologic protocols were used as measures of excitatory–inhibitory balance

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(15,16), and maximal dentate activation (kindling-like) protocols (17) were used to examine the effects of the KD on epileptogenesis. Some of these data were presented previously in abstract form (18).

METHODS

Animal feeding and measurement of ketosis

Male Sprague–Dawley rats (Animal Technologies Ltd., Kent, WA, U.S.A.; n = 36) were housed three to four to a cage and fed one of three diets: (a) NCR, (b) KCR, or (c) NAL diet. Administration of the KD was calorie-restricted (KCR) to 80–90% of the recommended daily allowance for rats (0.3 kcal/g body weight/day) (11,19). In addition to a control group fed NAL, a cohort of rats maintained on an NCR diet was tested to evaluate the effects of calorie-restriction (CR) alone. CR diets were administered in quantities to make both KCR and NCR diets calorically equivalent. Water was provided ad libitum throughout all experiments. A detailed description of the constituents of the normal (Purina 5001) and ketogenic (Bio-Serv F3666, Frenchtown, NJ, U.S.A.) diets has been reported previously (20). All animals began diet treatment at postnatal day 37–39 (P37–P39). CR animals were fasted 6–8 h before the initiation of either NCR or KCR treatment, and were fed individually once each day for 2–3 h, beginning between 12:00 and 15:00 h. Diets were maintained for $\geq$28 days before electrophysiologic testing. Although other metabolic indicators may help predict treatment outcome (e.g., glucose, with its "antiketogenic" potential), only plasma levels of β-hydroxybutyrate (BHB) were measured in this study. Blood was collected via tail clips at the end of each electrophysiologic experiment and assayed immediately by using a Keto-Site reflectance meter (GDS Technologies, Elkhart, IN, U.S.A.).

Electrophysiology

Rats were anesthetized with urethane (1.2–1.5 g/kg, i.p.) and placed into a stereotaxic apparatus. Body temperature was maintained on a thermal heating pad at 37°C. Holes were drilled in the skull, and electrodes were directed toward the dentate gyrus (recording position) and angular bundle (stimulating position). A bipolar, concentric stimulating electrode (SNEX 100; David Kopf Instruments, Tujunga, CA, U.S.A.), angled at 5 degrees toward midline, was positioned so that its tip rested at 1.0 mm anterior to lambda, 4.5 mm from the midline, and 2.0 mm down from the surface of the brain. Glass recording microelectrodes (~7–10 MΩ) were filled with 2 *M* NaCl and positioned ipsilaterally: 4.0 mm posterior to bregma, 2.0 mm from the midline, and 1.8 mm from the surface of the brain. Both stimulating and recording electrode depths were optimized to maximize the dentate population spike amplitude. Somewhat different stereotaxic coordinates were used for NAL-fed rats because body weight for ad libitum animals was always greater than those maintained on either CR diet. For these animals, the stimulating electrode was positioned at 7.6 mm posterior to bregma, 4.6 mm from the midline, and 2.2 mm from the surface of the brain. The recording electrode was positioned ipsilaterally at 4.6 mm posterior to bregma, 2.6 mm from the midline, and 2.2 mm down from the brain surface; electrode depths were again optimized. In all experiments, the animal was grounded through a subcutaneous Ag/AgCl wire placed intrascapularly.

Twenty minutes after electrode placement, single stimuli (0.05 Hz, 150-μs pulse duration) were administered to determine population spike (PS) threshold (T). Threshold was defined as the smallest stimulus intensity for which a dentate PS could be elicited. Stimulus intensities were standardized to multiples of T (e.g., 1 × T, 1.5 × T) and used for subsequent analyses.

Input/Output

Input/output (I/O) curves were constructed to measure differences in tissue excitability (within the dentate gyrus) between KD- and ND-fed rats. I/O relations were obtained by measuring the PS amplitude and slope of the field excitatory postsynaptic potential (fEPSP) from the first response to a pair of pulses (750-ms interpulse interval), delivered at 0.1 Hz, over increasing stimulus intensities. The PS amplitude was measured from the peak negativity of the spike to the point at which a vertically drawn line would intersect a line drawn between the two positive maxima of the response (15); the fEPSP slope (also measured at the level of the granule cell bodies) was determined from linear regression analysis of the 10–90% rising phase of the fEPSP before the onset of the PS. For a given animal, the PS amplitude and fEPSP responses to a given stimulus intensity were calculated from the average of at least five individual responses, and plotted as a percentage of the maximal response elicited.

Paired pulse

Paired pulses were delivered to assess inhibition or facilitation in the dentate gyrus network (15). Stimuli were delivered at 0.1 Hz (Master 8; AMPI, Jerusalem, Israel), a frequency suggested to activate primarily feedback circuitry (21). Recordings were amplified (AxoProbe 1A; Axon Instruments) and stored on computer for subsequent analysis (pClamp; Axon Instruments, Union City, CA, U.S.A.). PS amplitudes were normalized to the maximal amplitude of the first pulse (of each pair of responses) for a given IPI and stimulus intensity. PS ratios were calculated by dividing the mean amplitude of the second spike (of the pair) by the mean amplitude of the first spike. Amplitude ratios >1 were indicative of paired-pulse facilitation, whereas ratios <1 reflected inhibition. Measurements ±3 standard deviations from the group mean were disregarded, and group means were compared statistically by analysis of variance (ANOVA) with post hoc tests on ranks (SigmaStat; SPSS, Chicago, IL, U.S.A.).

Maximal dentate activation

Twenty minutes after paired-pulse experiments, maximal dentate activation (MDA) experiments were begun. Three variables were measured in these experiments: (a) the stimulus intensity required to produce an initial MDA event (i.e., MDA threshold); (b) the time-to-onset of successive MDA events; and (c) the MDA duration (including afterdischarge duration) with successive MDA events.

MDA threshold

Stimulus trains (pulses of 0.3-ms duration, at 20 Hz) were delivered at an initial intensity of 0.2 × T, and MDA was characterized electrophysiologically by (a) the onset of large-amplitude PSs (i.e., 20–40 mV), and (b) a rapid negative DC shift in baseline (22). If MDA was not elicited at 0.2 × T, the stimulus intensity was increased by increments of 0.1–0.2 × T and redelivered every 2.5 min until MDA was induced. The stimulus train was discontinued 2–5 s into MDA for all subsequent trains of stimuli such that subsequent paroxysmal discharges in the dentate gyrus were seen as afterdischarges rather than intrastimulus responses (23). Thus an afterdischarge was defined as any paroxysmal activity occurring after termination of the stimulus train (Fig. 6).

Time-to-onset and duration of MDA

Both time-to-onset and duration of MDA depend on stimulus intensity (17). Therefore, after MDA threshold values were initially determined, additional stimulus trains of increasing intensities (+0.1–0.2 × T) were administered at 5-min intervals to determine reproducible "plateau" levels from which time-to-onset and MDA duration analysis could be based, as previously described (23). Once "steady-state" values were reached, stimulus intensities were no longer increased, and the time-to-onset and duration of MDA were measured as repeated, seizure-inducing trains were delivered every 10 min for the next 4 h (total of 24 stimuli).

As shown in Fig. 6, the latency to MDA onset was measured from stimulus onset to the midpoint of the abrupt downward (negative) DC potential shift. The duration of MDA was measured from the midpoint of this negative shift in DC potential to the midpoint of its return to baseline. To assess time-to-onset and duration of MDA after repeated trains of stimuli, (i) the overall change in the group mean (±SEM) over time was compared by repeated-measures ANOVA (Sigma Stat), and (ii) the "rate" of change was assessed by calculating the slopes of regression lines for each animal and comparing group means with ANOVA with post hoc tests on ranks (SigmaStat). After spreading depression (SD) events, parameters of MDA were markedly affected such that time-to-onset and duration of MDA increased and shortened, respectively. Therefore, to permit comparisons between the "unaffected" CR diet groups and NAL-fed controls (see results below), only stimuli 1–15 were considered for our MDA analysis. Measurements ±3 standard deviations from the group mean were disregarded, and group means were compared statistically with ANOVA with post hoc tests on ranks (SigmaStat; SPSS). Differences were considered significant at $p < 0.05$.

RESULTS

Ketonemia and body weight

Ketonemia has been clinically considered a key feature of successful KD treatment (8). To ensure that ketonemia had been induced in animals fed a KD, blood plasma levels of BHB were measured for all groups. We found that BHB levels (±SEM) for animals maintained on the KCR diet (1.51 ± 0.11 m*M*) were markedly greater than those in animals fed the NCR diet (0.38 ± 0.04 m*M*), which in turn were significantly greater than those in animals maintained on the NAL diet (0.28 ± 0.06 m*M*; $p < 0.001$, ANOVA with Tukey). Body weights for NAL- and NCR-fed animals increased over the course of diet treatment (NAL: from 144 ± 3 to 285 ± 6 g; NCR: from 149 ± 5 to 155 ± 4 g; $p < 0.05$, paired *t* test), whereas KCR-fed animals lost weight (from 145 ± 4 to 136 ± 3 g; $p < 0.05$, paired *t* test). No correlations were found between body weight and ketonemia for any of the diet groups.

Input–output curves

As previous work showed that the KD can be anticonvulsant in rats (11,12,24), we measured I/O relations for fEPSP slope and PS amplitude to assess differences in synaptic excitability between groups. As shown in Fig. 1A, a significant, rightward shift occurred in the stimulus intensity necessary to produce a half-maximal PS for either of the CR groups (NCR $ES_{50} = 1.84\ \mu A$; 95% CI, 1.7–1.9 μA; KCR $ES_{50} = 2.0\ \mu A$; 95% CI, 1.95–2.19 μA) compared with NAL-fed animals ($ES_{50} = 0.53\ \mu A$; 95% CI, 0.47–0.58 μA). At stimulus intensity of 1 mA, this shift resulted in a more than threefold increase in PS amplitude for NAL-fed animals; NALs exhibited a mean PS amplitude of 8.8 ± 0.8 mA (94% of max) compared with a PS amplitude of 2.3 ± 0.4 mA (13% of max) for NCRs or 1.1 ± 0.1 mA (8% of max) for KCRs ($p < 0.05$; ANOVA with Tukey; Fig. 1C). Interestingly, the maximal PS amplitude also differed significantly across groups, with the NAL animals exhibiting a much lower plateau level than the NCR and KCR rats, and KCR animals exhibiting somewhat lower maximal amplitudes than NCR rats (NAL, 9.44 ± 0.8 mV; NCR, 18.14 ± 1.9 mV; KCR, 13.77 ± 1.3 mV; $p < 0.05$, ANOVA with Tukey; Fig. 1B).

A rightward shift also was seen in the I/O curve for CR-fed animals when the slope of the fEPSP was measured. Animals maintained on the NCR ($ES_{50} = 0.78\ \mu A$; 95% CI, 0.61–0.94 μA) and KCR diets ($ES_{50} = 0.90\ \mu A$; 95% CI, 0.77–1.0 μA) required significantly greater stimulus intensities to evoke half-maximal fEPSP slopes than did

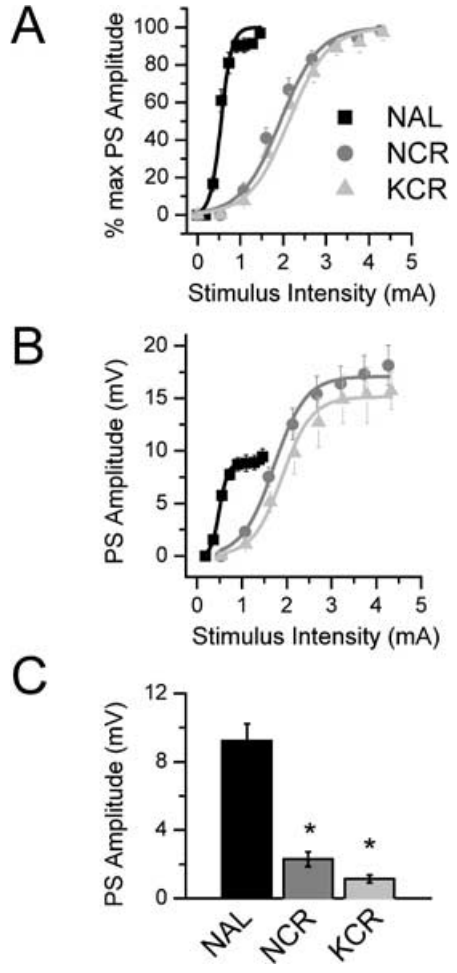

FIG. 1. Calorie restriction differentially affects neuronal excitability. **A:** A significant rightward shift in population spike (PS) amplitude (input/output curves for normal, calorie-restricted (NCR, n = 11, *dark-gray line*) and ketogenic, calorie-restricted (KCR, n = 10, *light-gray line*) was seen compared with normal, ad libitum–fed (NAL, n = 11, *black line*) controls, suggesting diminished neuronal excitability within the dentate gyrus. **B:** This also was apparent when PS amplitude (rather than percentage of maximal PS amplitude) was graphed, although it is clear that the NAL animals exhibited a significantly lower maximal PS amplitude compared with CR animals [$p < 0.05$, analysis of variance (ANOVA) with Tukey]. **C:** Note the more than threefold reduction in PS amplitude for CR animals when the stimulus intensity was at 1 mA ($p < 0.05$, ANOVA with Tukey). Values represent means ± SEM.

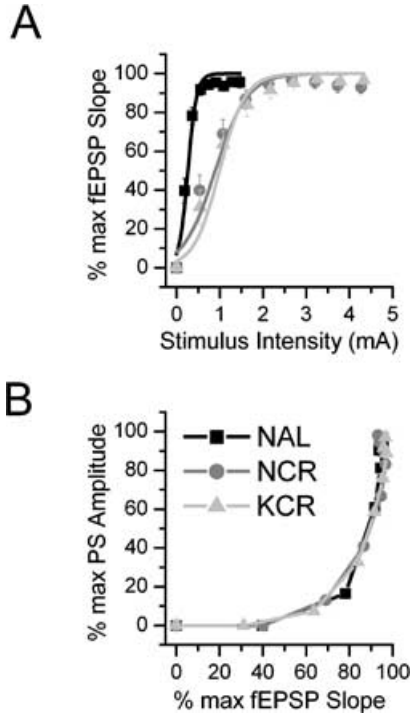

FIG. 2. A: Input/Output analysis of the field excitatory postsynaptic potential (fEPSP) slope also showed a rightward shift for CR-fed animals versus ad libitum–fed controls. **B:** fEPSP-spike (E-S) coupling, however, was not significantly altered with diet treatment. Error bars are ± SEM.

NAL-fed animals ($ES_{50} = 0.23$ μA; 95% CI, 0.19–0.28 μA; Fig. 2A). Field EPSP–spike (E-S) coupling, however, was similar for all three groups (Fig. 2B).

Paired-pulse inhibition

Previous studies have shown that treatment with the KD can increase the resistance to seizures both electrically (11) and chemically (20,24,25). We used paired-pulse protocols to test the hypothesis that functional inhibition within the dentate gyrus network was, therefore, augmented. Stimuli were delivered at interpulse intervals (IPIs) of 30, 70, 150, 250, 500, and 750 ms to evaluate fast inhibition (30 ms), facilitation (70 ms), and slow inhibition (250 ms) (26). We found both CR groups showed significantly greater paired-pulse inhibition (PPI) compared with NAL-fed animals at the 30-ms IPI ($p < 0.005$, ANOVA with Dunn's), whereas no differences were found at any of the other IPIs tested (Fig. 3). Further, the increase in inhibition at the 30-ms IPI remained significantly augmented in CR animals over varying stimulus intensities (Fig. 4); for all tested intensities greater than threshold-level stimulation, CR-fed animals maintained markedly lower paired-pulse ratios compared with NAL-fed controls ($p < 0.05$, ANOVA with Dunn's).

Maximal dentate activation and electrographic seizures

Both clinical (8) and experimental (27,28) studies have suggested that the KD might be antiepileptic as well as anticonvulsant. Therefore we also investigated whether the KD could inhibit the progressive prolongation of kindling-like afterdischarges in a model of epileptogenesis (i.e., MDA). Initially, threshold stimulation intensities required to produce MDA were established for each animal (Fig. 5A). Both CR treatments significantly elevated MDA threshold compared with those in NAL-fed controls ($p < 0.05$; ANOVA with Tukey); no statistical differences were seen between either of the CR diet groups. Neither body weight nor ketonemia were found to correlate to

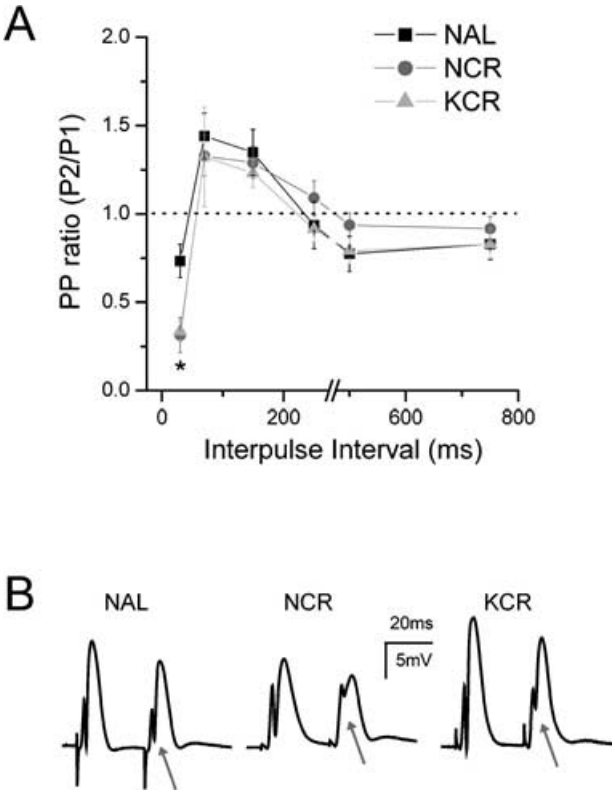

FIG. 3. Animals fed calorie-restricted (CR) diets exhibit greater paired-pulse inhibition than ad-libitum controls. **A:** Mean paired-pulse values for normal, calorie-restricted (NCR; n = 11), ketogenic, calorie-restricted (KCR; n = 9), and normal, ad libitum (NAL; n = 11) are shown for different interpulse intervals (IPIs) at 0.1 Hz. CR animals exhibited greater fast inhibition at the 30-ms IPI than did NAL-fed controls [$p < 0.05$ analysis of variance (ANOVA) with Tukey]; no other differences were noted for other IPIs tested ($p > 0.05$, ANOVA with Tukey). Stimulus intensity was 2 × T. Error bars are ± SEM. **B:** Sample traces from each of the diet groups highlight the enhancement of fast paired-pulse inhibition as recorded at the 30-ms IPI. Stimulus intensity was 2 × T. Note the difference in size of the second spike (*gray arrows*) compared with the first across diet groups.

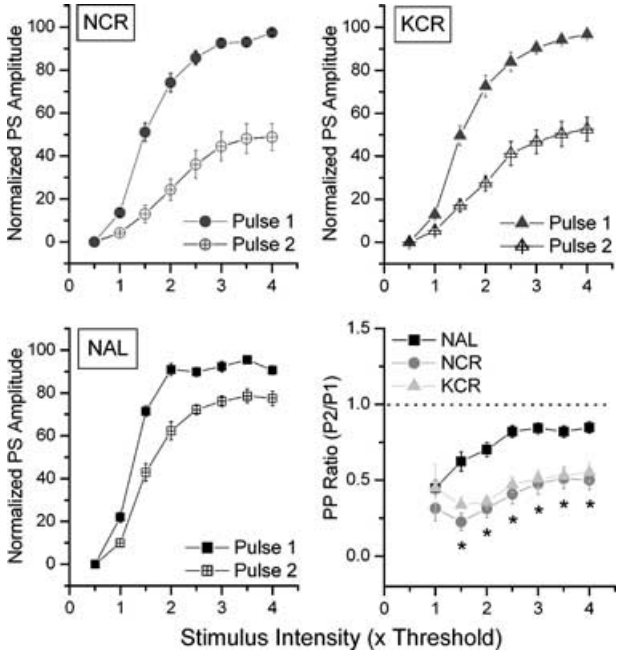

FIG. 4. Paired-pulse inhibition at the 30-ms interpulse interval (IPI) is maintained with increasing stimulus intensity at 0.1 Hz. There is a greater inhibition of the second population spike (PS) in calorie-restricted (CR) animals (**upper**) than there was in ad libitum controls (**lower left**). This translated to a significant reduction of the paired-pulse ratio (Pulse 2/Pulse 1) for normal, calorie-restricted (NCR; n = 11) and ketogenic, calorie-restricted (KCR; n = 9) groups compared with normal, ad libitum animals (NAL; n = 11) ($p < 0.01$, analysis of variance with Dunn's). Values are plotted as means ± SEM.

MDA threshold for any of the diet groups, with one exception; interestingly, there was a positive correlation between ketonemia and MDA threshold for NCR-fed animals ($r^2 = 0.62$, $p < 0.05$).

Two other aspects of MDA also were measured and compared between treatment groups: latency to MDA onset and MDA duration. Latency to MDA onset was significantly increased in NCR- and KCR-fed groups versus NAL-fed controls ($p < 0.02$, ANOVA with Tukey); no statistical differences occurred between the two CR diet groups ($p = 0.19$, ANOVA with Tukey). Over subsequent, seizure-inducing stimuli, the latency to onset decreased for all diet treatment groups ($p < 0.01$, repeated measures ANOVA). Importantly, however, the "rate of change" (i.e., decline in slopes) for MDA onset did not change, suggesting the anticonvulsant effects of CR persisted over successive stimuli (Fig. 5B; $p > 0.05$, ANOVA with Tukey on slopes of linear regression lines). The duration of MDA (including the afterdischarge duration) also was measured after repetitive seizure induction. We found that MDA/afterdischarge duration was dramatically prolonged in NCR- and NAL-fed animals compared with animals from the KCR group (Fig. 6). Although it was found that all diet groups exhibited a mean increase in MDA duration over time (Fig. 7; $p < 0.05$, repeated measures ANOVA), the rate at which the MDA duration increased was markedly reduced for KCR-treated animals compared with either NCR- or NAL-fed controls ($p < 0.05$, ANOVA with Dunn's on slopes of linear regression lines), and no difference was found in the rate at which MDA duration increased for NAL- versus NCR-fed animals ($p = 0.36$, ANOVA on slopes of linear regression lines).

As SD has been considered an indication of network hyperexcitability (29), it is noteworthy that SD events were observed in NAL-fed animals only (Fig. 8A). Episodes of SD first appeared between stimulations 16 and 20, when the MDA duration approached 20 s. By stimulation 24 (i.e., 4 h after onset of MDA stimulation), every NAL-fed animal exhibited at least one SD event (six of six). These SD events were never observed in either NCR- (none of seven) or KCR-fed (none of nine) rats. NAL-fed rats exhibited an average of 1.5 ± 0.34 SD events

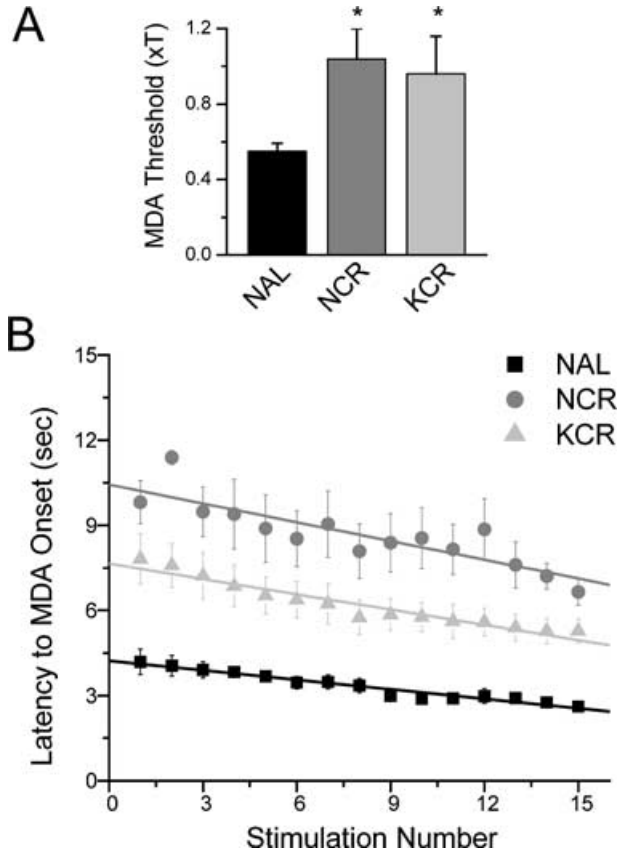

FIG. 5. Calorie-restricted (CR) diets are anticonvulsant. **A:** Animals maintained on either a normal, calorie-restricted (NCR; n = 10) or a ketogenic, calorie-restricted (KCR; n = 11) diet exhibit a greater threshold to maximal dentate activation (MDA) than do normal ad libitum (NAL; n = 11)–fed animals [$p < 0.05$, analysis of variance (ANOVA) with Tukey]. Values depicted are means ± SEM. **B:** Despite a reduction in the latency to MDA onset over the course of repetitive seizure-inducing stimuli ($p < 0.05$, repeated measures ANOVA), NCR-fed (n = 7) and KCR-fed (n = 9) animals maintain an elevated resistance to electrographic seizures over NAL (n = 5) controls ($p < 0.05$, ANOVA with Tukey).

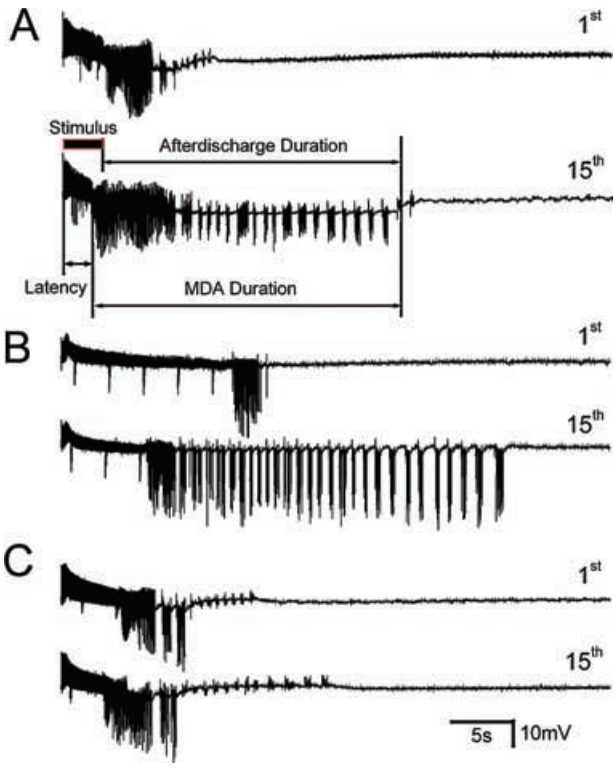

FIG. 6. Maximal dentate activation (MDA) duration is not prolonged in ketogenic, calorie-restricted (KCR; n = 9) animals as compared with either normal, calorie-restricted (NCR; n = 7) or normal ad libitum (NAL; n = 6) controls after 15 repetitive seizure-inducing stimuli. Note the difference between the first stimulation (1^{st}) and fifteenth stimulation (15^{th}) for animals maintained on the **(A)** NAL, **(B)** NCR, or **(C)** KCR diet. Parameters of MDA were measured as indicated in the lower panel of **A**.

(in 24 stimuli), a significant increase above either of the CR groups (Fig. 8B; $p < 0.05$, ANOVA with Tukey).

DISCUSSION

In this study, anticonvulsant and antiepileptic effects of the calorie-restricted, ketogenic diet were investigated in vivo. We found that CR shifted I/O curves to the right, increased paired-pulse inhibition at the 30-ms IPI, increased the resistance to electrographic seizures, and prevented SD events. These findings suggest that CR, perhaps through an augmentation of fast inhibition, is responsible for the anticonvulsant actions of the diet. In the MDA model of epileptogenesis, however, only animals maintained on the KCR diet were resistant to a "kindling-like" prolongation of electrographic seizure duration. From this result, we conclude that a KCR diet, in addition to its anticonvulsant effect, might also be antiepileptogenic.

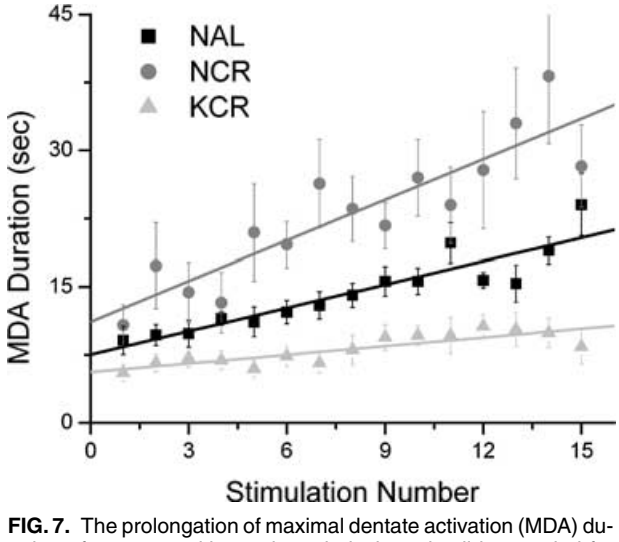

FIG. 7. The prolongation of maximal dentate activation (MDA) duration after 15 repetitive, seizure-inducing stimuli is retarded for ketogenic, calorie-restricted (KCR; n = 9) animals compared with either normal, calorie-restricted (NCR; n = 7) or normal ad libitum (NAL; n = 6) controls ($p < 0.05$, analysis of variance on slopes of regression). Values are presented as means ± SEM.

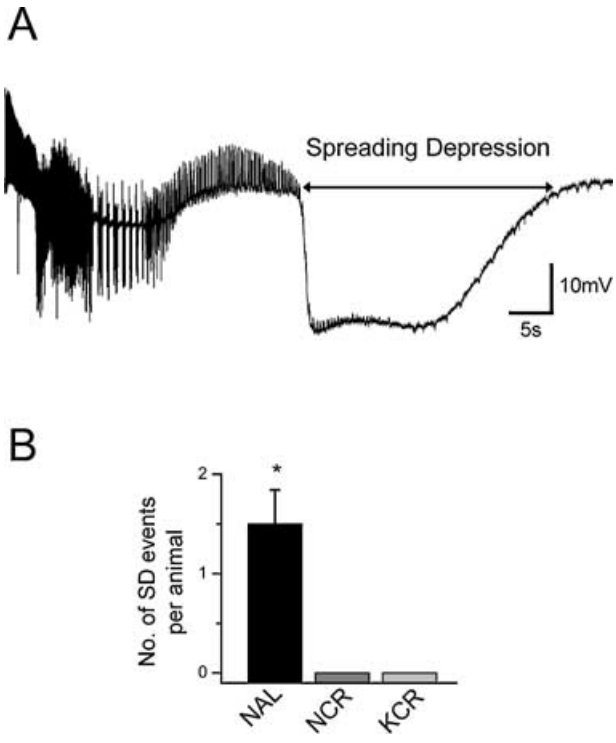

FIG. 8. Spreading depression (SD) was observed only in ad libitum controls and never observed in animals maintained on calorie-restricted (CR) diets. **A:** A sample trace showing a spreading depression event in a normal, ad libitum (NAL; n = 6) control. This SD event occurred on the sixteenth MDA stimulation. **B:** NAL-fed animals, on average, exhibited significantly more SD events than did normal, calorie-restricted (NCR; n = 7) or ketogenic, calorie-restricted (KCR; n = 9) animals ($p < 0.05$, analysis of variance with Tukey). Values are expressed as means ± SEM.

Diet-induced changes in neuronal excitability

Drugs that increase synaptic excitability (e.g., pilocarpine) have been shown to shift I/O curves to the left (30), whereas drugs that dampen synaptic excitability (e.g., CNQX and D-AP5) shift the I/O curve to the right (31). The rightward shift noted for the NCR and KCR diet groups (Fig. 1A and B; Fig. 2A) suggests that synaptic excitability is diminished in rats maintained on CR diets; the likelihood that a cell will generate an action potential from a given synaptic drive, however, remained unchanged (Fig. 2B). Because E-S coupling was overlapping between groups, these results indicate that the observed reduction in neuronal excitability can be attributed entirely to the diminution of the fEPSP slope and are consistent with a decreased probability of excitatory neurotransmitter release from presynaptic neurons. This conclusion is complicated, however, by the observation of much larger maximal PS amplitudes in the CR groups compared with NAL controls (Fig. 1B). At high stimulus intensities, the population of cells available to contribute to the PS is much larger in the CR groups, suggesting that a general enhanced inhibition in these animals can be overcome by sufficiently intense excitatory drive. Whether such high stimulus levels are "physiologically relevant" is unclear.

We subsequently measured the level of PPI within the dentate. In a previous in vitro study, hippocampal slices taken from KD-fed animals failed to show any differences in synaptic excitability compared with controls (32); no differences occurred in either I/O curves or paired-pulse relations (at any of the IPIs tested, 15–1,000 ms). In the present study, however, we observed changes in both I/O curves (Figs. 1 and 2) and paired-pulse relationships (Fig. 3). Given that the reintroduction of carbohydrates can result in rapid loss of seizure control in KD-treated patients (7,8), the standard slice medium (containing 10 m*M* glucose) might be expected to alter the biochemical milieu associated with ketosis and reverse any KD-induced change in excitability. These findings highlight potential difficulties in investigating the effects of the KD in vitro and suggest that CR can augment inhibition.

Pharmacologic studies conducted in vivo have shown that muscimol and diazepam [a γ-aminobutyric acid $(GABA)_A$ receptor agonist and allosteric modulator, respectively] enhance paired-pulse inhibition, whereas bicuculline (a $GABA_A$-receptor antagonist) diminishes paired-pulse inhibition (15,33). For each of these GABAergic modulators, only fast IPIs (i.e., <100 ms) were affected. Kapur et al. (33) concluded that diazepam- and muscimol-induced increases in paired-pulse inhibition reflected potentiation of fast GABAergic (i.e., $GABA_A$-mediated) inhibition in the hippocampus. Moreover, high concentrations of ketone bodies have been reported to increase GABA synaptosomal content in vitro (34), and elevated concentrations of GABA have been reported in forebrain and cerebellar homogenates of KD-fed mice (35). Although the present extracellular studies provide only initial indirect evidence, our results (Fig. 3) are consistent with these studies suggesting that both CR diet treatments act to enhance fast, GABAergic inhibition (via $GABA_A$ receptors) within the dentate gyrus network. Further experiments (e.g., analyses of IPSCs), however, will be necessary to confirm this hypothesis.

Diet-induced effects on electrographic seizures

Numerous studies have characterized measurable aspects of MDA and described its potential use as a tool to study different aspects of limbic seizure activity (22,36–38). It has been suggested that the latency to onset of MDA can be used as a gauge of seizure threshold; the duration of MDA can be used to measure of processes that terminate seizure activity in the limbic system; and the rate of increase in MDA duration has been described as a paradigm analogous to electrical kindling.

Both clinical (2,4) and experimental (9–12) studies have clearly indicated that the KD treatment increases the resistance to seizures. From our work, CR diets increased MDA threshold compared with those in NAL-fed controls

(Fig. 5A). Similarly, the latency to MDA onset remained significantly elevated above NAL controls (Fig. 5B). These findings not only show that the KD is anticonvulsant, but also indicate that CR, by itself, can increase the resistance to electroconvulsive seizures. Further, because KD also is CR, these data suggest that the anticonvulsant actions of the diet may be attributable to CR.

In addition to its noted anticonvulsant actions, clinical and experimental reports have suggested that the KD and CR might also be antiepileptogenic (8,27,28,39). In view of the similarities between CR groups in the present experiments (Figs. 1–5 and 8), it was expected that both the NCR and KCR treatments alike might prevent the prolongation of MDA duration. However, we found that only the KCR diet inhibited the progressive prolongation of "kindling-like" electrographic seizure discharges after repeated MDA (Figs. 6 and 7). Given that CR (15–30%) has been positively correlated to antiepileptogenic effects in EL mice (39), perhaps the 10–20% CR in the present study was not sufficient for the animals we tested, and a greater degree of CR (e.g., 15–30%) might have retarded/inhibited the rate of increase in MDA duration to a similar degree as observed for KD-fed animals.

It also may be possible that the "antiepileptogenic" interpretation for the KCR diet can be attributed to an enhanced anticonvulsant effect (compared with the NCR diet). Indeed, animals maintained on the KCR diet exhibit a higher PTZ seizure threshold than do NCR- or NAL-fed rats (40). Thus a greater anticonvulsant action of the KCR diet might have inhibited the prolongation of MDA by preventing electrographic seizure expression (compared with the lesser anticonvulsant action of the NCR diet). However, in light of our observations that the anticonvulsant effects for both CR diet treatments remained equivalent over repeated MDA stimulations (Fig. 5), it does not seem likely that the lack of prolongation of MDA duration exhibited by the KCR group could be ascribed entirely to an anticonvulsant mechanism of action.

Finally, it is important to note that MDA is a short-term protocol spanning only 1 day. It is improbable that the chronic structural changes (e.g., mossy fiber sprouting, neuronal cell loss, reactive gliosis) observed in other models of epileptogenesis (i.e., kainic acid, pilocarpine, kindling) would occur during a 1-day time frame. Nevertheless, repetitive MDA stimulations have been shown to produce not only acute hyperexcitability (41,42), but also long-term (2–4 weeks) reactive gliosis (43), and mossy fiber sprouting (44). Inasmuch as (i) AED treatments that inhibit the lengthening of evoked afterdischarges (analogous to MDA) have been considered antiepileptogenic (45), and (ii) KD treatment reduces mossy fiber sprouting (27), chronic hyperexcitability (32), and the frequency of spontaneous recurrent seizures (27,28,32), we consider the inhibition of MDA prolongation (Figs. 6 and 7) to be consistent with an antiepileptogenic role for the KD.

In summary, this work shows that the CR, as well as KD, can be anticonvulsant, and suggests that CR and KD likely act via a common mechanism of action: augmentation of fast inhibition. If KD is simply a more effective manner to induce and maintain a physiologic state of CR, then the putative antiepileptogenic effects described for KD-fed animals might also be found with greater CR. It also is intriguing that ketone bodies (as produced by CR and, to a greater extent, KD) can limit neuronal degeneration in the in vitro models of Alzheimer and Parkinson disease (46), and CR has been shown to increase the resistance of hippocampal neurons to kainate-induced excitotoxicity (47). As such it will be interesting to see whether CR (and KD?) treatment might also be useful in treating a wide variety of neurodegenerative processes other than just those occurring in the epileptic brain.

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