Anticonvulsant and Antiepileptic Actions of 2-Deoxy-D-Glucose in Epilepsy Models

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Objective: Conventional anticonvulsants reduce neuronal excitability through effects on ion channels and synaptic function. Anticonvulsant mechanisms of the ketogenic diet remain incompletely understood. Because carbohydrates are restricted in patients on the ketogenic diet, we evaluated the effects of limiting carbohydrate availability by reducing glycolysis using the glycolytic inhibitor 2-deoxy-D-glucose (2DG) in experimental models of seizures and epilepsy.

Methods: Acute anticonvulsant actions of 2DG were assessed in vitro in rat hippocampal slices perfused with 7.5mM $[K^+]_o$, 4-aminopyridine, or bicuculline, and in vivo against seizures evoked by 6Hz stimulation in mice, audiogenic stimulation in Fring's mice, and maximal electroshock and subcutaneous pentylenetetrazol (Metrazol) in rats. Chronic antiepileptic effects of 2DG were evaluated in rats kindled from olfactory bulb or perforant path.

Results: 2DG (10mM) reduced interictal epileptiform bursts induced by 7.5mM $[K^+]_o$, 4-aminopyridine, and bicuculline, and electrographic seizures induced by high $[K^+]_o$ in CA3 of hippocampus. 2DG reduced seizures evoked by 6Hz stimulation in mice (effective dose [ED]50 = 79.7mg/kg) and audiogenic stimulation in Fring's mice (ED50 = 206.4mg/kg). 2DG exerted chronic antiepileptic action by increasing afterdischarge thresholds in perforant path (but not olfactory bulb) kindling and caused a twofold slowing in progression of kindled seizures at both stimulation sites. 2DG did not protect against maximal electroshock or Metrazol seizures.

Interpretation: The glycolytic inhibitor 2DG exerts acute anticonvulsant and chronic antiepileptic actions, and has a novel pattern of effectiveness in preclinical screening models. These results identify metabolic regulation as a potential therapeutic target for seizure suppression and modification of epileptogenesis.

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Approximately 0.5 to 1% of people are afflicted with epilepsy, and as many as one third of patients with epilepsy are refractory to pharmacological therapy, including the most recent generation of anticonvulsants. The high-fat, low-carbohydrate, adequate-protein ketogenic diet (KD) has proven efficacy in reducing seizures in up to half of drug-refractory patients.[1] The mechanisms by which the KD suppresses seizures are largely unknown.[2,3] A remarkable feature of the KD is that ingestion of even a small amount of carbohydrate by patients who have achieved seizure control on the diet can rapidly reduce the diet's effectiveness and result in seizure recurrence.[4] This clinical observation suggests that glycolysis and carbohydrate metabolism may promote seizure susceptibility, and that inhibition or reduction of glycolysis may have anticonvulsant effects. In support of this hypothesis, preliminary in vitro observations demonstrated that isomolar substitution of glucose by alternative energy sources such as pyruvate and lactate in hippocampal slices exposed to 7.5mM $[K^+]_o$ reduced interictal epileptic burst discharges.[5] Furthermore, administration of 2-deoxy-D-glucose (2DG), an inhibitor of glycolysis, had in vivo anticonvulsant effects and reduced the progression of kindling evoked by perforant path stimulation in adult rats.[6]

2DG differs from normal glucose only by removal of an oxygen atom from the hydroxyl group at the 2 position (Fig 1A). When provided exogenously, 2DG is taken up by glucose transporters and is subsequently phosphorylated to 2-deoxy-D-glucose 6-phosphate (2DG-6P), which cannot be converted to fructose-6-phosphate by phosphoglucose isomerase, thereby preventing metabolism through subsequent steps of glycolysis (see Fig 1B). The trapping of 2DG-6P in cells after uptake through glucose transporters has enabled

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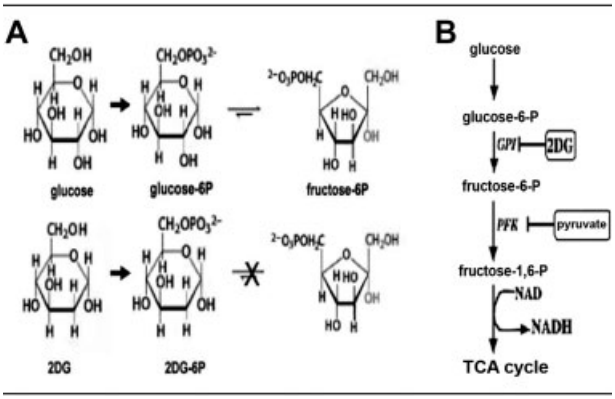

Fig 1. (A) Chemical structures of glucose (Glu), 2-deoxy-D-glucose (2DG), and intermediates of the initial steps of glycolysis. Phosphorylation of 2DG yields 2DG-6P, which cannot undergo isomerization by glucose-6-phosphate (glucose-6P) isomerase (GPI) to fructose-6-phosphate (fructose-6P), thereby preventing subsequent steps of glycolysis. (B) Schematic diagram of key steps of glycolysis illustrating the rate-limiting step involving phosphofructokinase, which is inhibited by pyruvate, the end product of the pathway. Oxidation of phosphoenolpyruvate (structure not shown) to pyruvate generates nicotinamide adenine dinucleotide (NADH) before entry into the tricarboxylic acid (TCA) cycle.

the use of 2DG as a metabolic tracer for glucose utilization and its adaptation in positron emission tomography scanning using ^{18}F-2DG in clinical imaging.[7]

In our previous study, intraperitoneal administration of 2DG at a dose of 250mg/kg in rats impaired the development of kindled seizures in response to stimulation of the perforant path to the dentate gyrus and hippocampus.[6] The antiepileptic effects of 2DG against kindling were associated with reduction of seizure-induced increases in brain-derived neurotrophic factor (BDNF) and its receptor trkB, which are required for kindling progression.[8] Seizure-induced increases in BDNF and trkB were prevented by 2DG through a mechanism of transcriptional repression dependent on neuron-restrictive silencing factor (NRSF) and its NADH sensitive cofactor carboxy-terminal binding protein (CtBP), which maintain a repressive chromatin environment at the BDNF and trkB promoter regions.[6] These observations suggest that reducing glycolytic flux by glycolytic inhibitors such as 2DG may have anticonvulsant and antiepileptic effects.

In this study, we further evaluated the acute anticonvulsant properties of 2DG against epileptic burst discharges evoked in vitro in rat hippocampal slices and in in vivo models including seizures evoked by maximal electroshock (MES), subcutaneous pentylenetetrazol (Metrazol), 6Hz stimulation in mice, and audiogenic stimuli in Fring's mice. The chronic effects of 2DG against epilepsy progression were also examined by investigating the effects of 2DG on development of kindled seizures evoked by stimulation of a different neural structure, the olfactory bulb.

Materials and Methods

Protocols for the experiments reported here were approved by the University of Wisconsin Research Animal Resources Center (RARC) and conform to animal care guidelines of the National Institutes of Health.

Hippocampal Slice Experiments

Sprague–Dawley male rats (10–13 or 28–120 days old) were deeply anesthetized with isoflurane. After decapitation, brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl 124, KCl 3.75, NaH_2PO_4 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, glucose 10, and $CaCl_2$ 2 saturated with a mixture of 95% oxygen and 5% CO_2. The pH of all solutions was adjusted to 7.4. Horizontal hippocampal slices (500μm) were cut using a Vibratome series 1000 tissue slicer (Vibratom, St. Louis, MO) and were maintained at room temperature for at least 1 hour, after which they were transferred to an interface recording chamber with ACSF maintained at 32°C for electrophysiological recordings.

Extracellular field potentials were recorded in stratum pyramidale of CA3 using a glass pipette filled with 2M NaCl (impedances of 5–10MΩ). Spontaneous activity was recorded, amplified, stored using a DIGIDATA 1200 AD converter and Axoclamp 1B amplifier, and analyzed with pCLAMP 6.02 (Axon Instruments Inc., Union City, CA). After baseline recording, epileptiform burst discharges were induced by increasing $[K^+]_o$ to 7.5mM,[9,10] or bath addition of the convulsant agents 4-aminopyridine (50–100μM) or bicuculline (10μM). There is a long controversy regarding the semantics and phenomenology of seizure activity in in vitro brain slices.[11] Strictly speaking, behavioral seizures (the defining feature of clinical epilepsy) cannot occur in a brain slice. Here we use the terminology outlined in comprehensive literature reviews,[11,12] defining brief (typically <100 milliseconds), interictal-like discharges as epileptiform discharges or epileptiform bursts, and longer (several seconds, presumably ictal) seizure-like discharges as electrographic seizures.[9,10,13,14]

After sufficient recording to determine that the rate of spontaneous epileptic discharges was at steady state (usually about 30 minutes), effects of alterations in the concentration of glucose, bath additions of alternative energy sources such as pyruvate or lactate, or effects of bath addition of 2DG were evaluated.

Seizures Evoked by Maximal Electroshock in Rats

MES seizures or convulsions were evoked in rats (adult Sprague–Dawley male rats weighing 100–150gm) by 60Hz alternating current (150mA) delivered for 2 seconds by corneal electrodes primed with an electrolyte solution containing an anesthetic agent (0.5% tetracaine HCl). Effects of 50 to 200mg/kg 2DG orally (PO) against MES convulsions were evaluated at 15 minutes to 4 hours after administration. Animals were considered protected from MES-induced seizures on abolition of the hind-limb tonic extensor component of the seizure.[15]

Seizures Evoked by Subcutaneous Metrazol in Rats

Subcutaneous administration of 70mg/kg pentylenetetrazole (Metrazol) reliably induces convulsions with features of clonic spasms of limbs, jaws, and vibrissae in 97% of rats (adult Sprague–Dawley male rats weighing 100–150gm) (convulsive dose or CD_{97}: 70mg/kg). Clonic spasms of approximately 3 to 5 seconds of the fore and/or hind limbs, jaws, or vibrissae were the end points, and animals that did not meet this end-point criterion were considered protected. Rats were pretreated with 200 to 400mg/kg 2DG intraperitoneally (IP) or 50 to 200mg/kg 2DG PO 30 minutes before administration of Metrazol for assessment of dose response. Rats received 50 to 200mg/kg 2DG IP for determination of time course of action against Metrazol-induced seizures at 1 to 24 hours after administration of 2DG. The time course of action of 30mg/kg 2DG PO was evaluated at 30 minutes and 1 hour after administration.

Audiogenic Seizures in Fring's Mice

Fring's mice reliably exhibit seizures with tonic limb extension after exposure to a 110dB, 11kHz sound stimulus. Adult Fring's mice (18–25gm) were placed into a round Plexiglas sound chamber and exposed to 110dB, 11kHz sound for 20 seconds or until full tonic extension was noted. Animals not demonstrating tonic extension were considered protected. Dose response was determined by exposure to the sound stimulus at 15 minutes after administration of 2DG at a dose of 125 to 250mg/kg IP. Time to peak effect was determined by exposure to the sound stimulus at 15 minutes to 2 hours after administration of 2DG at a dose of 125 to 250mg/kg IP.

6Hz Seizures in Mice

Seizures were induced in male albino mice via corneal stimulation (6Hz, 0.2-millisecond rectangular pulse width, 3-second duration at an intensity of 22mA) applied with 0.5% tetracaine corneal anesthesia.[16] Seizures were characterized by stun, forelimb clonus, twitching of the vibrissae, and Straub tail. Protection was defined as the absence of a seizure without toxicity. Dose response was determined by assessment of protection at 15 minutes after administration of 2DG at a dose of 15 to 200mg/kg IP. Time to peak effect was determined by assessing protection at 15 minutes to 4 hours after administration of 2DG at a dose of 75 to 100mg/kg IP.

Kindling Procedures in Rats

Kindled seizures were evoked by stimulation of the olfactory bulb in Sprague–Dawley male rats (250–350gm). Rats were anesthetized with ketamine (80mg/kg IP) and xylazine (10mg/kg intramuscularly), and implanted with an insulated stainless steel bipolar electrode for stimulation and recording. The electrode was implanted in the olfactory bulb (coordinates in relation to bregma: 0.9cm anterior, 0.12cm lateral, 0.18cm ventral) and was fixed to the skull with acrylic. A screw inserted into the skull served as ground. These rats were compared with another group of rats reported previously[6] that was implanted with electrodes in the perforant path (coordinates in relation to bregma: 0.81cm posterior, 0.44cm lateral, 0.35cm ventral) and otherwise treated identically. After a 1-week recovery period, both groups of unrestrained, awake, implanted rats received twice daily kindling stimulations (5 days per week) with a 1-second train of 60Hz, biphasic, constant current, 1-millisecond, square wave pulses as described later. The electroencephalogram was recorded from the bipolar electrode, which was switched to the stimulator for the delivery of kindling stimulations.

On the first day of stimulation, the afterdischarge threshold (ADT) was determined in each rat by delivery of a series of stimulus trains ranging from 100 to 1,100μA. If the initial stimulus intensity (100μA) evoked an afterdischarge (AD), that intensity was used in subsequent twice-daily stimulations. If an AD was not evoked by 100μA, the stimulation current was increased sequentially to 200, 300, 400, 500, 700, 900, 1,000, or 1,100μA, until an AD was evoked. If 1,100μA failed to evoke an AD, stimulation was continued on subsequent days at 1,100μA. The current intensity that initially evoked an AD was used for subsequent twice-daily stimulations and was systematically modified according to the following protocol to deliver stimulation at the lowest intensity required to evoke an AD. If an AD was evoked by three consecutive stimulations at a given intensity, the stimulation was decreased in 100μA decrements. If an AD was not evoked after three stimulations, the stimulation was increased by 100μA increments to a maximum of 1,500μA. If three stimulations at 100μA caused an AD, the stimulation was decreased to 70μA and then to 50μA.

Evoked behavioral seizures were classified according to standard criteria ranging from class I (behavioral arrest) to class V seizures (bilateral tonic-clonic motor activity with rearing and loss of postural tone),[17] which are comparable with human complex partial seizures with secondary generalization. The initial ADT, determined as described earlier, served as the baseline for comparison of the effects of repeated kindling stimulations on ADT and the effect of 2DG treatment. After the third evoked AD, rats were randomized to receive either 2DG 250mg/kg IP or an equal volume of normal saline IP 30 minutes before each kindling stimulation.

Analysis and Statistical Procedures

To examine the time course of 2DG effects on the kindling ADT and to allow intergroup comparisons, we divided stimulation intensities for each rat by the intensity required to evoke the baseline ADs and plotted them as a function of AD number. Rats received twice-daily kindling stimulations until at least three tonic-clonic seizures with secondary generalization (class V) were evoked. All data are presented as the mean ± standard error of the mean. Differences across groups were analyzed with the Tukey's and Holm–Sidak tests for analysis of variance or the Student's t test when appropriate. Differences with confidence levels $p < 0.05$ were considered significant.

Results

Anticonvulsant Effects of Glycolytic Inhibition on Epileptiform Bursting in Hippocampal Slices

To investigate the possibility that glucose and glycolysis contribute to the maintenance of epileptiform activity, we evaluated the effect of glucose withdrawal on inter-

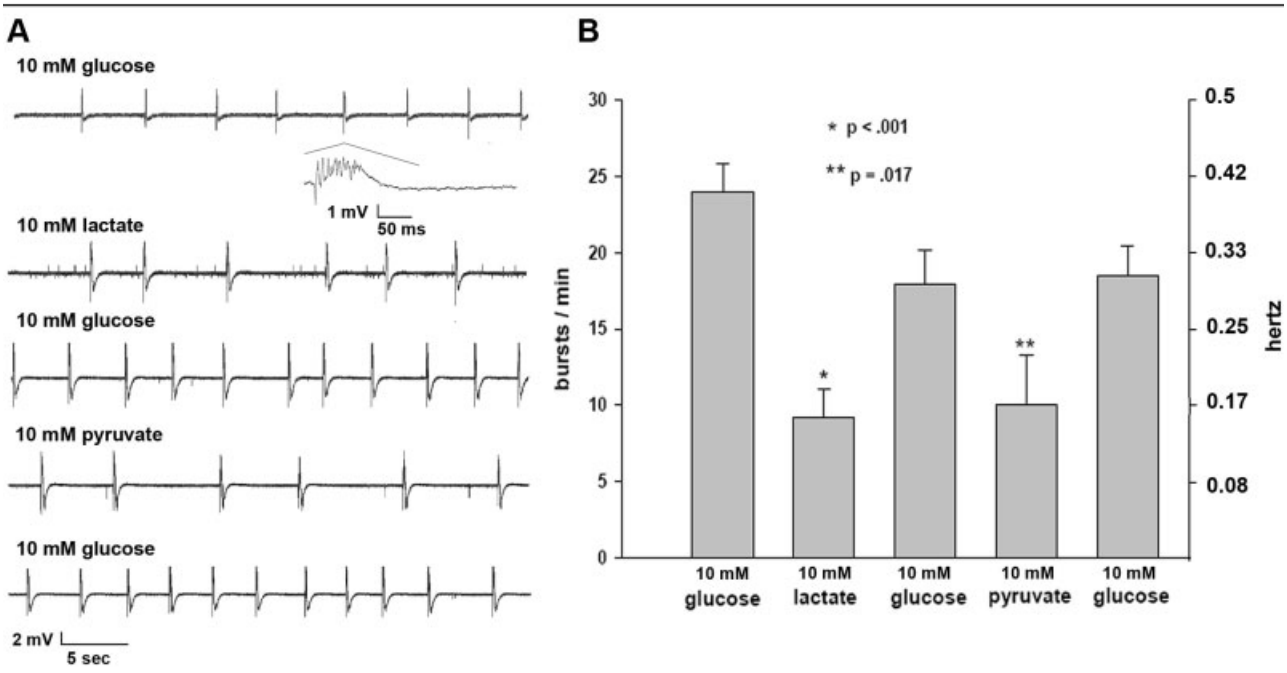

Fig 2. Anticonvulsant effects of removal of glucose and substitution with alternative energy sources lactate or pyruvate on interictal burst firing induced by 7.5mM $[K^+]_O$. (A) Extracellular recording of spontaneous interictal discharges in hippocampal area CA3 (1- to 4-month-old rats) in standard artificial cerebrospinal fluid (ACSF) with 10mM glucose. Faster sweep speed in the inset demonstrates that the discharges consisted of spontaneous extracellular depolarizations with superimposed population spikes. Removal of glucose and substitution with 10mM lactate reduced the frequency of interictal discharges. Washout of lactate and return to standard ACSF containing 10mM glucose increased interictal discharges, which were subsequently reduced by removal of glucose and substitution with 10mM pyruvate, a direct inhibitor of glycolysis. (B) Effects of removal of glucose and isomolar substitution of 10mM lactate or 10mM pyruvate as illustrated in (A) from recordings in 21 hippocampal slices from 8 rats. Asterisks *indicate significant differences: *p < 0.001; **p = 0.017.*

ictal burst frequency in the CA3 region of rat hippocampus. Hippocampal slices bathed in 7.5mM $[K^+]_o$ developed brief epileptiform bursts consisting of spontaneous, rhythmic, high-amplitude, extracellular, positive potentials with superimposed bursts of negative population spikes followed by a prolonged extracellular negativity consistent with interictal discharges (Fig 2A, also see inset). These bursts are defined here as interictal based on terminology used in previous literature.[10,11] After establishing the baseline burst firing rate during a period of 30 minutes of recording in 10mM glucose (approximately 24.0 ± 1.8 bursts/min), we altered the bathing solution by removal of glucose and isomolar substitution of 10mM lactate as an alternative energy source (see Fig 2A) for the tricarboxylic acid cycle via pyruvate. After 30-minute exposure to ACSF containing 10mM lactate with no added glucose, there was a 60% decrease in burst discharge rate to 9.2 ± 1.8 per minute (see Fig 2B; n = 21 slices from 8 rats). Removal of lactate and return to 10mM glucose in the ACSF restored the initial burst frequency, confirming that the reduction in burst discharges in 10mM lactate was not caused by reduced viability of the slices. When glucose was again removed from the ACSF and replaced with 10mM pyruvate, which directly inhibits phosphofructokinase and subsequent steps of glycolysis (see Fig 1B), burst frequency after 30 minutes decreased to 10.0 ± 3.3 per minute, and burst frequency again recovered after washout of pyruvate and reintroduction of 10mM glucose (see Figs 2A, B; n = 17 slices from 8 rats). These data indicate that reducing glycolysis has an anticonvulsant effect via decreased neuronal excitability by reducing high $[K^+]_o$-induced interictal epileptiform bursts. These results imply that compounds that inhibit glycolysis may also have an anticonvulsant effect.

To test this hypothesis, we added the glycolytic inhibitor 2DG to hippocampal slices bathed in 7.5mM $[K^+]_o$ and 10mM glucose. An example of bath application of 10mM 2DG is provided in Figure 3. Figure 3A shows an example of spontaneous epileptiform field bursts recorded in CA3 in 7.5mM $[K^+]_o$ and10mM glucose. Burst frequency was reduced at 30 minutes after addition of 10mM 2DG. At 30 minutes after washout (return to normal ACSF with 10mM glucose; see Fig 3A, bottom trace), the effects of 2DG are reversed. The overall effect of 2DG on interictal burst frequency is illustrated in Figure 3B. Burst frequency was reduced

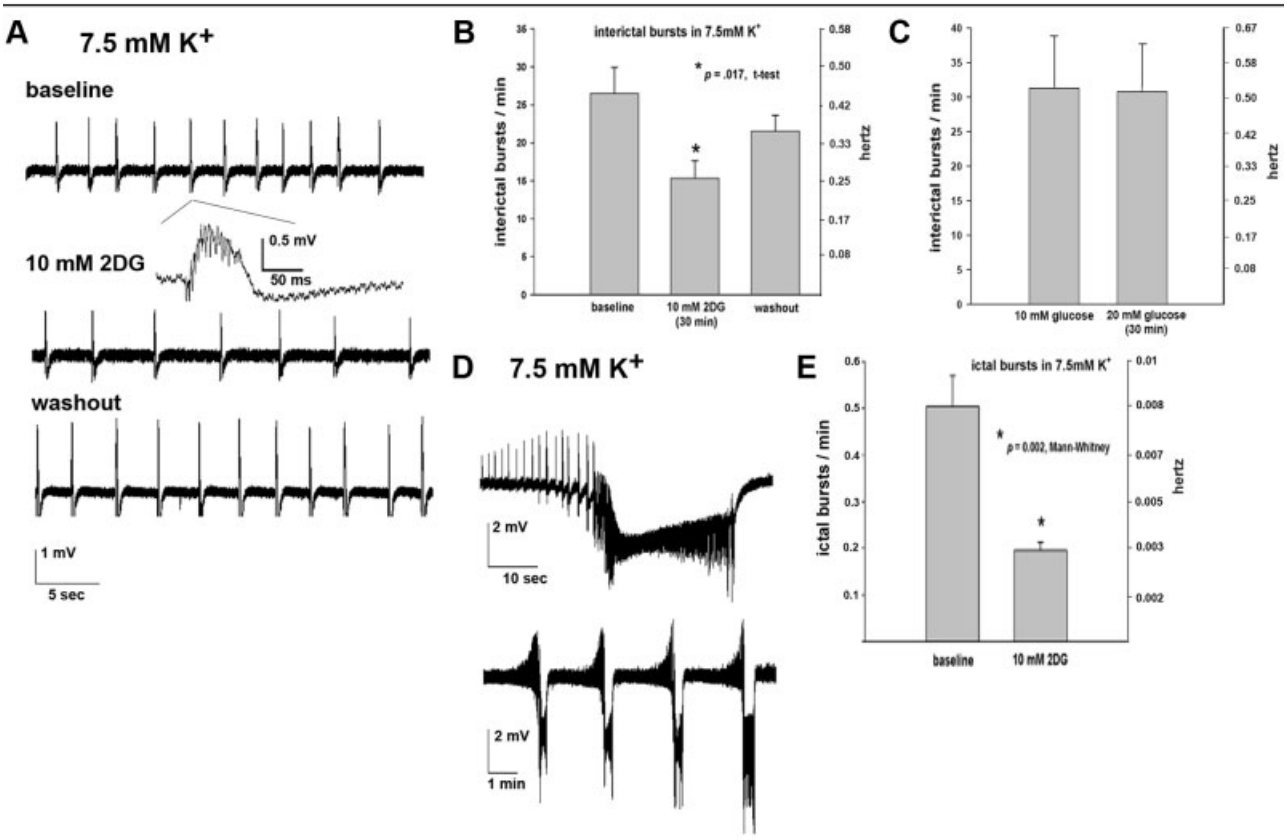

*Fig 3. Actions of 2-deoxy-D-glucose (2DG) on spontaneous interictal and ictal epileptiform bursts induced in hippocampal area CA3 by 7.5mM $[K^+]_O$. (A) Representative example of reversible effects of 10mM 2DG in reducing frequency of spontaneous interictal discharges. Inset shows an interictal burst at faster sweep speed. (B) Effects of 30 minutes of bath application of 10mM 2DG in 8 hippocampal slices from 5 rats. There was partial washout after return to standard artificial cerebrospinal fluid (ACSF). *p = 0.017, t test. (C) Effect of increasing bath concentration of glucose from 10 to 20mM. After 30 minutes of application of 20mM glucose, there was no change in interictal burst frequency (n = 5 slices from 3 rats). (D) Examples of spontaneous ictal discharges in CA3 consisting of a prolonged extracellular direct current shift with superimposed high-frequency spike discharges that were observed in a subset of hippocampal slices exposed to 7.5mM $[K^+]_o$. Slower sweep speed in bottom trace demonstrates rhythmicity of the spontaneous ictal discharges. (E) Anticonvulsant effect of 30-minute bath application of 10mM 2DG on ictal discharges (n = 7 hippocampal slices from 5 rats). *p = 0.002, Mann–Whitney U test. Interictal data (A–C) are from 1- to 4-month-old rats; ictal data (D, E) are from 10- to 13-day-old rats.*

by approximately 40% after bath application of 10mM 2DG (26.5 ± 3.5 to 15.3 ± 2.3 per minute; n = 8 slices from 5 rats). Although partial washout was observed after removal of 2DG from the ACSF, burst frequency remained reduced at 60 minutes after bath application, suggesting that the effects of 10mM 2DG may be long lasting. Bath application of lower concentrations of 2DG (1mM, 5mM) had no effect on interictal burst frequency, even 1 hour after bath application (data not shown). To determine whether the reduction in interictal burst frequency was caused by an action of 2DG, not simply by the effects of increased extracellular osmolality, we increased the bath concentration of glucose to 20mM for 30 minutes; there was no effect on burst frequency (see Fig 3C; n = 5 slices from 3 rats; $p = 0.966$, t test), excluding a role of increased osmolality in the reduction of burst frequency.

In the CA3 region of hippocampal slices from young rats, increased $[K^+]_o$ produces longer spontaneous events of neural firing defined as electrographic seizures.[10,11] In our experiments, these ictal-like discharges occurred in a subset of slices exposed to 7.5mM $[K^+]_o$ from 10- to 13-day-old rats, and consisted of prolonged high-frequency discharges superimposed on an extracellular field negativity. They developed after a series of briefer interictal epileptiform burst discharges increased in amplitude. An example of an ictal burst discharge recorded from CA3 in a hippocampal slice exposed to 7.5mM $[K^+]_o$ (from a 10-day-old rat) is illustrated in Figure 3D. Ictal discharges were reliably reduced by bath exposure to 10mM 2DG

(0.5 ± 0.07 per minute to 0.2 ± 0.02 per minute in 10mM 2DG; $p = 0.002$; see Fig 3E; n = 7 slices from 5 rats). Although CA3 ictal discharges can decrease over time ("run down"),[18] our observations are sufficiently numerous and stable to suggest a reproducible effect. Washout did not abate ictal discharges.

Bath application of 10mM 2DG also reduced interictal burst discharges in CA3 of hippocampal slices (from 1- to 4-month-old rats) exposed to the $GABA_A$ receptor antagonist, bicuculline (10μM). Interictal epileptiform burst discharges were reduced by approximately 60% after 30-minute bath application of 10mM 2DG, and suppressive effects of 2DG persisted after washout (Figs 4A, B; n = 9 slices from 4 rats). Interictal bursts in CA3 in hippocampal slices (from 1- to 4-month-old rats) exposed to the potassium channel blocker 4-aminopyridine (50–100μM) were also reversibly decreased by 30 minutes of bath application of 10mM 2DG (see Figs 4C, D; n = 13 slices from 6 rats).

Overall, these results demonstrate that 2DG suppresses interictal epileptiform discharges and ictal electrographic burst discharges induced in vitro in the hippocampal CA3 region by a variety of convulsant mechanisms. Although some observations have suggested that reduced activity in CA3 is associated with ictal activity in the disrupted circuitry of parahippocampal slices,[19,20] the suppressive effects of 2DG against both interictal and ictal local network synchronization in CA3 are consistent with an overall anticonvulsant effect in this region.[21]

Effects of 2-Deoxy-D-Glucose on Seizures Evoked by 6Hz Stimulation in Mice

Protection against 6Hz evoked seizures was observed at 15 minutes after administration of 2DG at doses of 75mg/kg IP in six of eight rats, after a dose of 200mg/kg IP in five of eight rats, and after a dose of 300mg/kg in 6 of 8 rats (Fig 5A). The calculated dose of 2DG administered IP that resulted in protection for 50% of rats (ED50) was 79.7mg/kg. The time to peak action of 2DG was 15 minutes at a dose of 75mg/kg IP and 1 hour at a dose of 100mg/kg IP.

Effects of 2-Deoxy-D-Glucose on Audiogenic Seizures in Fring's Mice

2DG protected against audiogenic seizures in four of eight mice at 2 hours after administration of 220mg/kg IP and in seven of eight mice after 250mg/kg for a calculated ED50 of 206.4mg/kg (see Fig 5B). The time to peak effect was 2 hours at both of these doses.

Effects of 2-Deoxy-D-Glucose on Seizures Evoked by Metrazol in Rats

There was also some evidence suggestive of anticonvulsant activity of 2DG against seizures evoked by Metrazol (Table 1). Two of eight rats were protected against Metrazol-evoked seizures at 30 minutes after a 2DG dose of 200mg/kg IP, and three of eight were protected after administration of 400mg/kg 2DG IP. Four of 12 rats were protected at 30 minutes after a dose of 50mg/kg PO, but only 2 of 8 were protected at a dose of 100mg/kg PO, and only 1 of 8 after a dose of 200mg/kg PO. Although suggesting that 2DG may have some anticonvulsant activity against seizures evoked by Metrazol, the overall results were not sufficient to calculate an ED50 or a time to peak effect.

Effects of 2-Deoxy-D-Glucose on Seizures Evoked by Maximal Electroshock in Rats

There were no protective effects against MES in rats at 15 minutes to 4 hours after 2DG was administered at doses of 100 to 200mg/kg PO (Table 2).

Anticonvulsant and Antiepileptic Effects of 2-Deoxy-D-Glucose on Seizures Evoked by Kindling

In a previous report,[6] administration of 2DG at a dose of 250mg/kg IP 30 minutes before stimulation of the perforant path had anticonvulsant effects manifested by increases in the ADT. This finding contrasts with the gradual decrease in ADT in untreated animals. The anticonvulsant effects of 2DG were further assessed here by examining the effects of 2DG on the ADTs and progression of seizures evoked by kindling of the olfactory bulb. After initial determination of the ADT using the standardized protocol described earlier in Materials and Methods, trains of 60Hz stimulation were delivered twice daily to the olfactory bulb. After three ADs were evoked, the rats were randomized to groups that received either 2DG (250mg/kg IP) or saline IP 30 minutes before each kindling stimulation. The effects of 2DG were compared with the saline-injected control animals and with the previously reported groups of rats that experienced seizures evoked by kindling of the perforant path and treatment with 2DG using the same protocols. The ADTs across animals and groups were normalized by expressing the current required to evoke each AD as a percentage of the initial average current required to evoke ADs in each animal before randomization.

The mean ADTs for each group are plotted in Figure 6 as a function of the number of evoked seizures. The mean initial ADT for the first three evoked ADs for rats treated with 2DG was not significantly different than the ADT for saline-treated rats (766.7 ± 105.4 vs 516.7 ± 155.8μA for olfactory bulb, $p = 0.19$, t test; 693.3 ± 85.9 vs 866.7 ± 88.2 for perforant path, $p = 0.176$, t test). The mean ADT of rats treated with saline before delivery of kindling stimulation to the perforant path or olfactory bulb exhibited a gradual and persistent reduction as a function of the number of evoked seizures as expected during the progression of evoked kindled seizures. There was no ef-

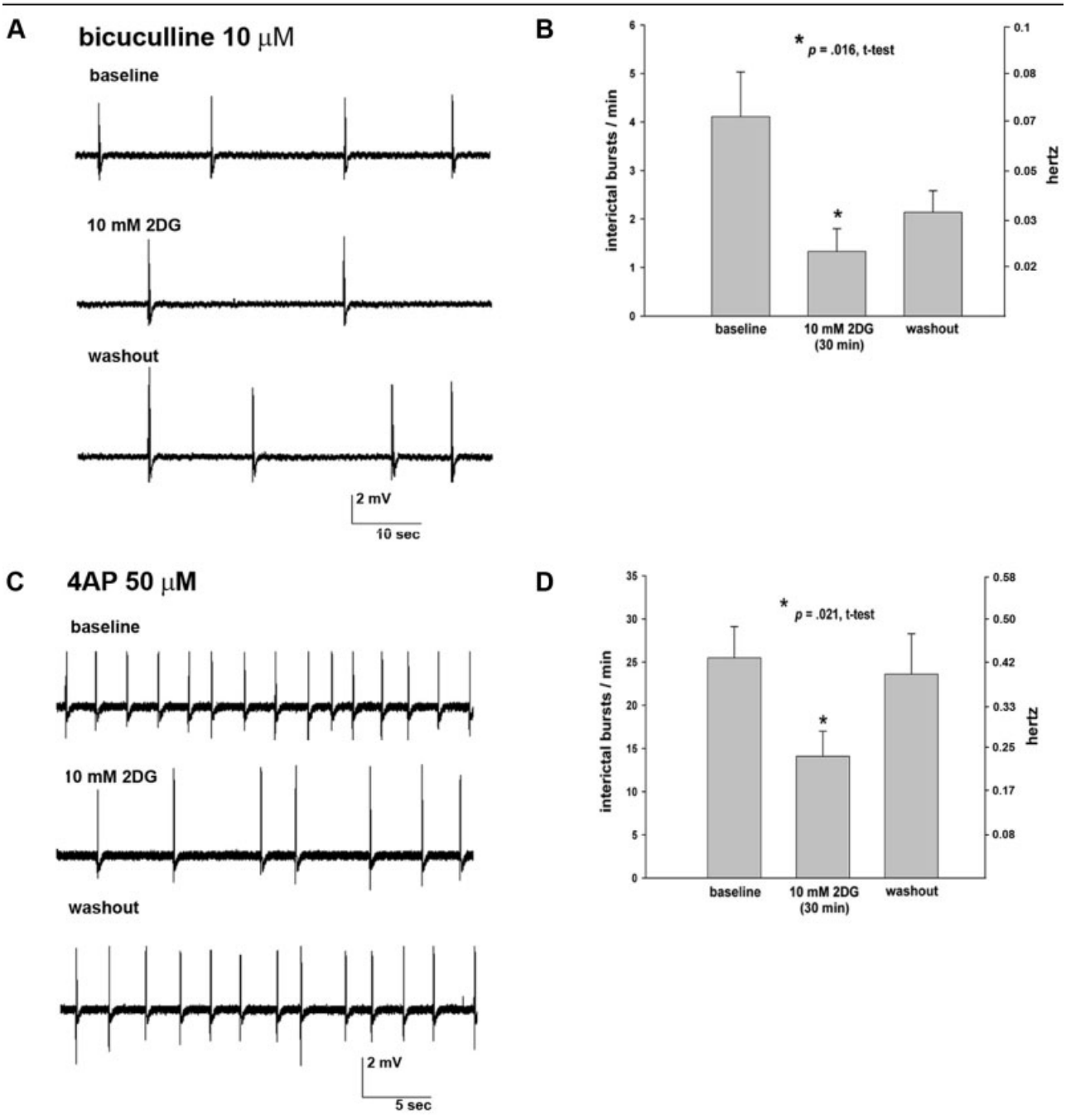

*Fig 4. Effects of 10mM 2-deoxy-D-glucose (2DG) on CA3 interictal bursts induced in hippocampal slices (from 1- to 4-month-old rats) by 10μM bicuculline or 50 to 100μM 4-aminopyridine (4AP). (A) Bath application of 10mM 2DG for 30 minutes reduced interictal burst discharges (middle trace) compared with baseline (upper trace). Bursts persisted after return to normal artificial cerebrospinal fluid (ACSF) (bottom trace). (B) Effects of 30-minute bath application of 10mM 2DG on interictal bursts induced by 10μM bicuculline (n = 9 hippocampal slices from 4 rats). *p = 0.016, t test. (C) Effects of 30-minute bath application of 10mM 2DG on interictal bursts induced by 50μM 4AP. Interictal burst frequency increased after washout of 2DG and return to normal ACSF (bottom trace). (D). Overall effects of 30-minute bath application of 10mM 2DG on interictal bursts induced by 50 to 100μM 4AP (n = 13 hippocampal slices from 6 rats). *p = 0.021, t test.*

fect of 2DG on the ADT of rats receiving kindling stimulation to the olfactory bulb, which contrasts with the increase in ADT in rats treated with 2DG on experiencing seizures evoked by kindling of the perforant path (see Fig 6). These results indicate that the anticonvulsant effect of 2DG on the ADT appears to be specific to the site of stimulation and suggest that the anticonvulsant mechanisms of 2DG may

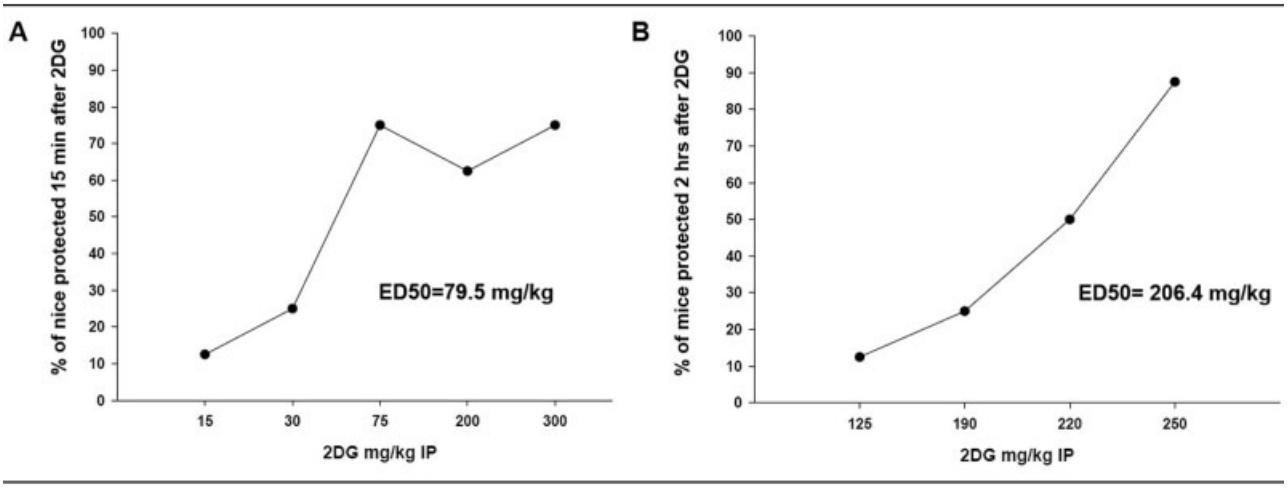

Fig 5. (A) Effects of 2-deoxy-D-glucose (2DG) on 6Hz seizures in mice. Percentage of mice protected at a stimulus of 22mA is shown as a function of intraperitoneal 2DG dose. The effective dose (ED) 50 is 79.7mg/kg. (B) Effects of 2DG on audiogenic seizures in Fring's mice. Percentage of mice protected 2 hours after 2DG administration is shown as a function of intraperitoneal 2DG dose. The ED50 is 206.4mg/kg.

Table 1. **Metrazol Seizures (number rats protected/tested)**

Subcutaneous Metrazol: Dose Response at 30 Minutes after Oral 2DG			
2DG dose	50mg/kg PO	100mg/kg PO	200mg/kg PO
Rats protected	4/12	2/8	1/8

Subcutaneous Metrazol: Time to Peak Effect after Oral 2DG		
Time of administration of 50mg/kg 2DG	30 min	1 hr
Rats protected	0/8	0/8

Subcutaneous Metrazol: Dose Response at 30 Minutes after Intraperitoneal 2DG		
2DG dose	200mg/kg IP	400mg/kg IP
Rats protected	2/8	3/8

Subcutaneous Metrazol: Time Course of Action of Intraperitoneal 2DG					
Time of administration of 2DG	2 hr	4 hr	6 hr	8 hr	24 hr
Rats protected (50mg/kg IP)	0/4	1/4	0/4	0/4	2/4
Rats protected (100mg/kg IP)	0/4	2/4	1/4	1/4	1/4
Rats protected (200mg/kg IP)	—	1/8	—	—	—

2DG = 2-deoxy-D-glucose; PO = orally; IP = intraperitoneally.

be differentially effective in particular regions of limbic circuitry.

Our previous study also showed that 2DG slowed of the rate of perforant path kindled seizure progression to the milestone of secondary generalized tonic-clonic (class V) seizures.[6] The number of evoked ADs required to reach the milestones of class III, IV, and V seizures is plotted in Figure 7 for both the previous perforant path data[6] and olfactory bulb stimulations. Rats treated with 2DG required approximately 50% more ADs evoked by stimulation of the olfactory bulb to reach the first class III or IV seizure or the first, second, or third class V seizure ($p = 0.001$; 2DG: n = 9; saline: n = 6). In rats treated with 2DG before kindling stimulation of the perforant path, the rate of achieving the milestones of class III, IV, and V seizures was similarly slowed as indicated by approximately 50% more ADs required to reach each of these stages (2DG: n = 15; saline: n = 12). There was no difference in the mean AD duration between 2DG-treated rats and saline-injected control rats in either the olfactory bulb or perforant path groups (data not shown), which implies that the reduced progression of seizures

Table 2. **Maximal Electroshock Seizures: Dose Response and Time Course of Action (number rats protected/tested)**

Dose	**15 min**	**30 min**	**1 hr**	**2 hr**	**4 hr**
100mg/kg 2DG PO	0/4	0/4	1/4	0/4	0/4
200mg/kg 2DG PO	—	—	0/8	—	—

2DG = 2-deoxy-D-glucose; PO = orally.

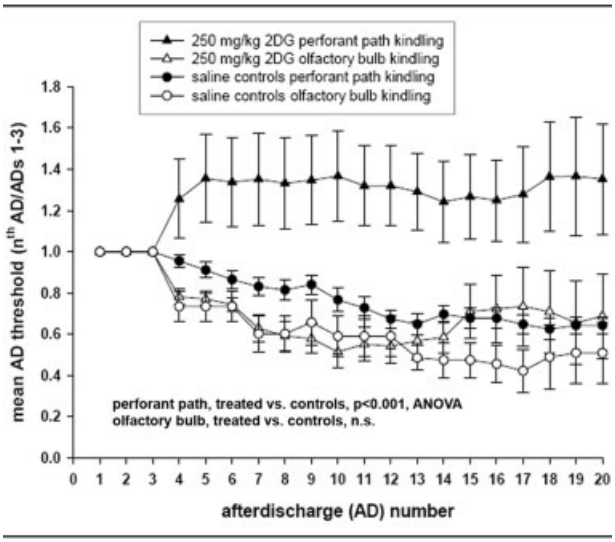

Fig 6. Effects of 2-deoxy-D-glucose (2DG) on afterdischarge (AD) threshold in rats experiencing kindled seizures evoked by stimulation of the olfactory bulb and perforant path. 2DG (250mg/kg intraperitoneally [IP]) was administered 30 minutes before delivery of stimulation. Treatment with 2DG increased the normalized mean AD threshold (filled triangles) *in rats receiving perforant path stimulation compared with saline-treated control animals, which demonstrated a reduction in AD threshold (*filled circles, *reported previously[6]). In contrast, there were no effects of 2DG on AD threshold of rats receiving stimulation of the olfactory bulb* (open triangles) *compared with saline-treated control animals* (open circles). *Perforant path: treated versus control animals,* $p < 0.001$, *analysis of variance; olfactory bulb: treated versus control animals,* p *value not significant.*

in the 2DG-treated groups was not merely a result of cumulative anticonvulsant effects of 2DG on ADT.

Discussion

These experiments demonstrate that 2DG, a glucose analogue used for decades as the positron emitting tracer ^{18}F-2DG for examination of regional glucose uptake, has acute anticonvulsant and chronic antiepileptic effects in a variety of in vitro and in vivo models of seizures and epilepsy. 2DG reduced the frequency of both interictal epileptiform bursts and ictal electrographic seizures induced by 7.5mM $[K^+]_o$ in the CA3 region of hippocampal slices, and also reduced interictal epileptiform bursts in CA3 evoked by bath application of 4-aminopyridine, a K^+ channel antagonist, and bicuculline, a $GABA_A$ receptor antagonist.

Inhibition of glycolysis may account for at least some of the reduction in neuronal excitability underlying the acute anticonvulsant action of 2DG in hippocampal slices, because blocking glycolysis by isomolar replacement of glucose with alternative energy sources such as pyruvate or lactate also suppressed epileptic discharges evoked by 7.5mM $[K^+]_o$.

In vivo, 2DG demonstrated chronic antiepileptic effects against kindling progression in response to stimulation of the olfactory bulb, confirming a previous observation of action against seizure progression by stimulation of the perforant path.[6] 2DG also acutely protected against seizures evoked in vivo by 6Hz stimulation in mice and against audiogenic seizures in Fring's mice. It showed only minimal evidence of anticonvulsant activity against seizures evoked by Metra-

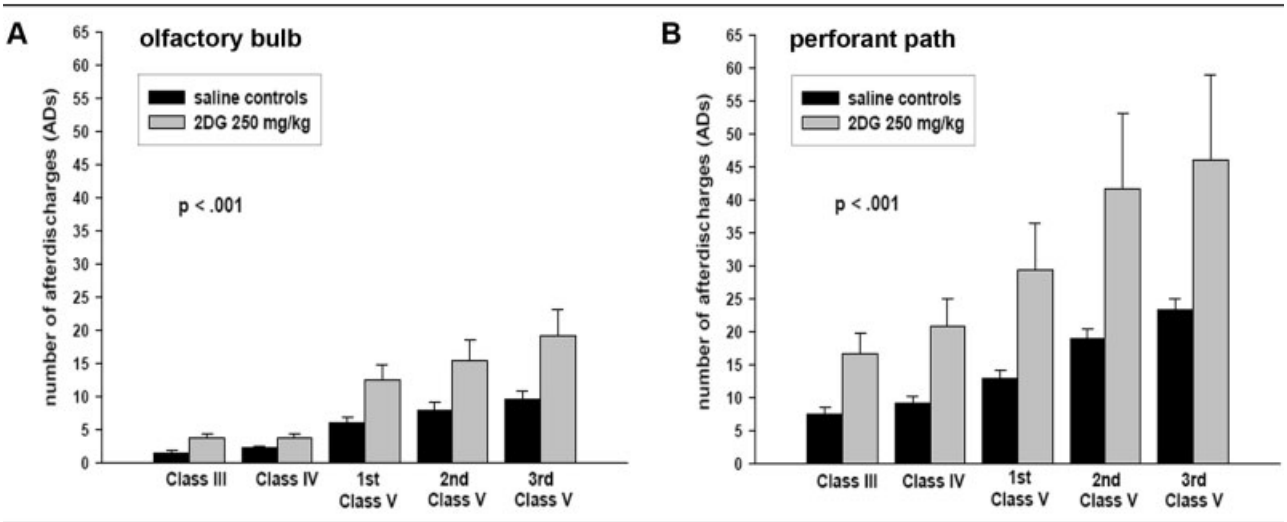

Fig 7. Antiepileptic effects of 2-deoxy-D-glucose (2DG; 250mg/kg intraperitoneally; gray bars*) on kindling progression in rats experiencing seizures evoked by perforant path or olfactory bulb stimulation. Number of afterdischarges (ADs) required to achieve behavioral seizure stages of class III, class IV, and the first to third class V seizures in response to kindling of the olfactory bulb (A) or perforant path (B). There was an approximately twofold slowing in the rate of number of ADs required to reach each stage in animals treated with 2DG for both olfactory bulb and perforant path stimulation.* $p < 0.001$. Black bars *represent saline control animals.*

zol and had no protective effects against MES seizures. These results provide evidence that 2DG has acute and chronic anticonvulsant and antiepileptic properties in several preclinical models. The spectrum of the effectiveness of 2DG against seizures evoked by 6Hz, audiogenic, and kindling stimulation is distinctive relative to other available anticonvulsants. Although the suppression of seizures by 2DG in these models was not complete, many well-established anticonvulsants do not confer complete protection or demonstrate effectiveness in all preclinical screening models. Partial but significant protection in various subsets of models implies potential for effectiveness in clinical trials in patients. Although none of the currently used screening models has been formally validated as reliable predictors of anticonvulsant action in people, there is consensus that identification of compounds with distinctive profiles of action in the models compared with available drugs is desirable and may be valuable for developing new drugs that are more effective than the currently marketed agents.[22–25] With this unique spectrum of protection in these animal models of epilepsy, 2DG has potential as a therapy for epilepsy with novel mechanisms of action compared with currently available anticonvulsants.

Acute In Vivo Anticonvulsant Actions of 2-Deoxy-D-Glucose

Protection against minimal clonic seizures evoked by 6Hz stimulation in mice, regarded as a model of "psychomotor" seizures,[16] was observed at 15 minutes to 1 hour after intraperitoneal administration. Anticonvulsant effects against audiogenic seizures in Fring's mice, which are generated, in part, by auditory circuitry in the brainstem, were observed at 1 to 2 hours after intraperitoneal administration. Anticonvulsant properties at these short-term intervals after in vivo administration are unlikely to be dependent on altered neuronal gene expression. Furthermore, 2DG had rapid acute onset of anticonvulsant action against interictal and ictal discharges in CA3 within minutes after bath application, suggesting that its acute anticonvulsant effects are likely to be operating at the membrane or synaptic levels, or possibly through alterations in second messenger pathways influenced acutely by metabolic effects of 2DG such as inhibition of glycolysis. As 2DG suppressed interictal and ictal events induced by distinctive mechanisms such as depolarization by increased $[K^+]_o$, blockade of potassium channels by 4-aminopyridine, and antagonism of $GABA_A$ receptors by bicuculline, its acute anticonvulsant actions appear to be broadly suppressive against a variety of cellular and membrane processes generating network synchronization.

Chronic In Vivo Effects against Progression of Kindled Seizures

These experiments confirmed that the previously reported antiepileptic effect of 2DG against progression of kindled seizures evoked by perforant path stimulation is also observed when kindled seizures are evoked by stimulation of the olfactory bulb. The rate of progression to the first evoked class V seizure was approximately doubled when 2DG was administered at 30 minutes before stimulation of either the perforant path or the olfactory bulb, indicating that 2DG interferes with neuronal mechanisms underlying seizure progression in limbic circuitry. Because temporal lobe epilepsy is the most common form of intractable drug-resistant epilepsy, 2DG could potentially modify the progressive course of seizure-induced alterations in limbic circuitry in that disorder.

Although protective effects of 2DG against progression of kindled seizures were observed with either perforant path or olfactory bulb stimulation, differences were observed as a function of the site of seizure induction. ADT was increased by 2DG in perforant path kindling but not in olfactory bulb kindling. The increase in the ADT in response to perforant path stimulation induced by 2DG is an anticonvulsant effect, because increase of the ADT indicates that seizure induction requires stimulation of increased intensity.[6] The ADT typically decreases as kindling progresses; therefore, the observation of an increase in the ADT in perforant path kindling indicates that 2DG modifies progressive seizure-induced plasticity that is normally a part of the kindling process. However, the anticonvulsant effect against ADT was not observed in the olfactory bulb, indicating that at least some of the effects of 2DG are region specific. The reasons for this difference are uncertain, but one possibility is that 2DG may have different effects on excitability or seizure-induced plasticity in the distinctive circuitries of the perforant path and olfactory bulb. None of the currently available conventional anticonvulsants demonstrates such regionally specific properties.

Possible Mechanisms Underlying the Acute Anticonvulsant and Chronic Antiepileptic Actions of 2-Deoxy-D-Glucose

The observations that 2DG acutely protects against seizures evoked by 6Hz stimulation in mice and audiogenic seizures in Fring's mice, and rapidly suppresses hippocampal interictal epileptiform bursts and electrographic seizures in vitro suggest that 2DG has acute anticonvulsant properties in addition to its chronic antiepileptic effects against kindling progression. These two actions could involve different cellular and molecular mechanisms.

The chronic antiepileptic effects of 2DG have been associated with repression of BDNF and trkB expres-

sion. Conditional knock-out of BDNF slowed kindling, and conditional knock-out of trkB blocked kindling progression.[8] The repression by 2DG of seizure-induced increases in BDNF and trkB is mediated by the transcriptional repressor NRSF and its NADH-sensitive corepressor CtBP acting at the promoter regions of BDNF and its receptor, trkB.[6] In pathophysiological conditions of high neuronal activity such as seizures, which increase glucose uptake and glycolysis, increases in NADH dissociate CtBP from NRSF and decrease repression, resulting in increased expression of BDNF and trkB. In the presence of 2DG, which reduces NADH levels as a consequence of glycolytic inhibition, the NRSF-CtBP complex maintains repression of BDNF and trkB, and kindling progression is slowed.

The rapid onset of anticonvulsant effects of 2DG suggest that this compound may be acting via different mechanisms, such as effects at the synaptic or membrane levels. Alternatively, the acute anticonvulsant actions of 2DG may be a result of unrecognized metabolic effects associated with glycolytic inhibition. For example, reduction of glycolysis may be accompanied by increases in systemic lipid metabolism, and alterations in activity of the Kreb's cycle and mitochondrial metabolism that could influence neuronal hyperexcitability. 2DG has been shown to increase neuronal resistance to oxidative and metabolic insults in cultured hippocampal neurons,[26] and to increase epileptic tolerance evoked by cerebral ischemia in mice.[27] Glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) maintain $GABA_A$ responses by phosphorylation of the $\alpha 1$ subunits of $GABA_A$ receptors.[28] Although GAPDH and glycolytic intermediates can influence $GABA_A$ receptors, the suppressive effects of 2DG on burst discharges in vitro and seizures in vivo suggest that glycolysis must have other acute effects on cellular and synaptic mechanisms contributing to network synchronization.

2-Deoxy-D-Glucose, the Ketogenic Diet, and Potential Anticonvulsant Effects of Altering Brain Energy Metabolism

This study was not intended to investigate cellular mechanisms underlying the KD, but rather to mimic some of the seizure-suppressive effects of the diet through modulation of glycolysis. Nevertheless, some of these findings may have relevance for the anticonvulsant action of the KD. Hypotheses for the KD mechanism of action include a direct or indirect effect of ketosis,[29] improvement in neuronal energy reserves,[30,31] enhancement of GABAergic inhibition,[32] alteration of mitochondrial metabolism,[33] upregulation of gene transcripts encoding energy metabolism enzymes,[34] and effects of lipids on neuronal excitability.[35,36] Clinical evidence suggests that carbohydrate restriction has beneficial effects, and that ingestion of even small amounts of carbohydrate can result in recurrence of seizures in patients who are well controlled on the KD.[4] The possibility that decreased carbohydrate (glucose) availability plays a role in the effects of the KD has also been considered.[37]

Glucose is an obligate energy source for the brain under normal conditions, but in situations of low glucose availability (eg, fasting or the high-fat, low-carbohydrate KD), other substances can provide the brain's energy requirements. Alternative energy sources that can maintain brain function and synaptic activity include lactate, pyruvate, and β-hydroxybutyrate.[38–42] Pyruvate is also a potent inhibitor of glycolysis by direct feedback inhibition of phosphofructokinase, the rate-limiting enzyme of glycolysis. In subjects on the KD, fatty acids are converted into ketone bodies (acetoacetate, β-hydroxybutyrate, acetone), and enter the brain via a monocarboxylate transporter.[43] These results and those of other studies implicate the reduction in carbohydrate availability or metabolism as feasible mechanisms for decreased neuronal excitability and enhanced seizure control.[37,44,45] The ability of calorie restriction to afford seizure protection in EL mice supports the hypothesis that reduction in carbohydrate metabolism may play a beneficial role in the KD.[46]

2DG does not model the KD because 2DG does not produce ketosis. However, 2DG and the KD do share some anticonvulsant effects. Both the KD and 2DG reduce audiogenic seizures in Fring's mice and in the 6Hz model.[47] The KD is effective in MES, whereas 2DG is not. 2DG and the KD are both effective in kindling, although experimental studies have utilized different protocols. In our experiments, ADT was determined by stimulation of the perforant path or olfactory bulb, followed by intraperitoneal administration of 2DG 30 minutes before each kindling stimulation. Another study used a "rapid" amygdala kindling protocol and monitored ADT in rats already fully kindled on either KD or standard diet.[48] Although the KD and 2DG are clearly different therapies, these results support carbohydrate restriction as a potential anticonvulsant and antiepileptic strategy.

2-Deoxy-D-Glucose Uptake, Metabolism, and Storage: Implications for Anticonvulsant Development

Glucose is taken up by neurons and glia, both of which possess uptake transporters and glycolytic enzymes that metabolize glucose for energy.[49–51] As an analogue of glucose, 2DG enters cells through glucose transporters, and its uptake is preferentially increased in cells with increased energy consumption and metabolic demands. This aspect of 2DG may be advantageous for enhanced delivery of 2DG in the specific regions of the brain that generate seizures. The activity-dependent uptake of 2DG in regions of brain with increased energy me-

tabolism also implies that pharmacodynamic actions (including anticonvulsant actions) and potential toxicity are likely to be nonlinear in relation to serum levels.

Although 2DG-6P does not undergo isomerization and progress through subsequent steps of glycolysis, glycogen becomes radiolabeled after injection of radiolabeled 2DG through isomerization to 2DG-1P and conjugation to uridin diphosphate UDP-2DG, 2DG glycogen, and 2DG glycoproteins.[52] As 2DG is incorporated into glycogen and glycosylated macromolecules presumably stored in astrocytes, it may be released when astrocytic glycogen is metabolized during states of high energy demand such as seizures. The possibility that 2DG may be stored for long periods in glycogen also raises questions about its potential for chronic toxicity.[53] However, 2DG has been administrated safely to humans for decades as the positron emission tomographic imaging tracer ^{18}F-2DG and has been administered to humans safely as adjuvant therapy for cancer,[54,55] suggesting that it has a relatively favorable preliminary toxicity profile for development as an anticonvulsant.

Conclusion

In summary, the experiments reported here demonstrate that 2DG has distinctive acute and chronic anticonvulsant therapeutic effects in preclinical in vivo models of seizures. The rapid onset of anticonvulsant suppressant action against burst discharges evoked in vitro by a variety of induction methods suggests that its anticonvulsant actions may be broad spectrum against a variety of mechanisms of network synchronization. The activity-dependent delivery of 2DG to brain regions with high metabolic activity, as occurs during seizures, further supports the clinical potential of this compound. Experiments are under way to further characterize the dose response and time course of anticonvulsant and antiepileptic actions of 2DG, and its effects on synaptic properties. Future experiments addressing the effects of 2DG on adenosine triphosphate–dependent K^+ currents or other ion channels influencing neuronal excitability are of potential interest for understanding how glycolytic metabolism affects seizures and may contribute to the therapeutic actions of the KD.

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