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Anticonvulsant and proconvulsant actions of 2-deoxy-D-glucose

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SUMMARY

Purpose: 2-Deoxy-D-glucose (2-DG), a glucose analog that accumulates in cells and interferes with carbohydrate metabolism by inhibiting glycolytic enzymes, has anticonvulsant actions. Recognizing that severe glucose deprivation can induce seizures, we sought to determine whether acute treatment with 2-DG can promote seizure susceptibility by assessing its effects on seizure threshold. For comparison, we studied 3-methyl-glucose (3-MG), which like 2-DG accumulates in cells and reduces glucose uptake, but does not inhibit glycolysis.

Methods: Mice were treated with 2-DG or 3-MG and the seizure threshold determined in the 6-Hz test, the mouse electroshock seizure threshold (MEST) test, and the intravenous pentylenetetrazol (i.v. PTZ) and kainic acid (i.v. KA) seizure threshold tests. 2-DG was also tested in fully amygdala-kindled rats.

Results: 2-DG (125–500 mg/kg, i.p., 30 min before testing) significantly elevated the seizure threshold in the 6-Hz seizure test. 2-DG (250–500 mg/kg) decreased the threshold in the MEST and i.v. PTZ and i.v. KA tests. 3-MG had no effect on seizure threshold in the 6-Hz test but, like 2-DG, decreased seizure threshold in the i.v. PTZ test. 2-DG (250 and 500 mg/kg, i.p., 30 min before testing) had no effect on amygdala-kindled seizures.

Conclusions: Although 2-DG protects against seizures in the 6-Hz seizure test, it promotes seizures in some other models. The proconvulsant action may relate to reduced glucose uptake, whereas the anticonvulsant action may require inhibition of glycolysis and shunting of glucose metabolism through the pentose phosphate pathway.

KEY WORDS: 2-Deoxy-D-glucose, 3-Methyl-glucose, Glucose metabolism, Glycolysis, Pentose phosphate pathway, Ketogenic diet, Kindling.

There is increasing interest in the use of dietary modifications to treat neurologic disorders (Gasior et al., 2006; Baranano & Hartman, 2008). One such dietary modification, the high fat, low carbohydrate ketogenic diet (KD), has gained acceptance for treating refractory epilepsy (Bailey et al., 2005; Freeman et al., 2007). No single mechanism has been identified to explain the seizure protection conferred by the KD (Hartman et al., 2007; Nylen et al., 2009). Mild hypoglycemia, significant ketosis, and a shift in cellular metabolism wherein ketone bodies replace glucose as the primary carbon source in the Krebs cycle represent the most fundamental metabolic changes produced by the KD. However, the exact roles of glucose and ketone bodies in brain metabolism and how alterations in glucose and ketone bodies impact seizure susceptibility are poorly understood.

Because the consumption of carbohydrates during the KD can lead to the rapid relapse of seizures (Huttenlocher, 1976) and different diets with carbohydrate levels below that of the typical American diet are also effective in the management of intractable epilepsy, altered glucose metabolism has been implicated as a key factor in the anticonvulsant efficacy of the KD (Pfeifer & Thiele, 2005; Freeman et al., 2006; Kossoff et al., 2006). This concept has inspired studies to determine if approaches that specifically decrease glucose utilization through the glycolytic pathway can also produce anticonvulsant effects (Garriga-Canut et al., 2006; Lian et al., 2007). Therefore, the glucose analog 2-deoxy-D-glucose (2-DG), which interferes with glucose uptake into cells and prevents glucose catabolism, has been found to reduce epileptiform discharges in vitro and to protect

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Preliminary findings of this study were presented during the 60th annual meeting of the American Epilepsy Society, San Diego, December 1–5, 2006. M.G.’s current address is Clinical Research, Cephalon, Inc., 41 Moores Road, Frazer, PA 19355, U.S.A.; e-mail: mgasior@cephalon.com.
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against seizures in several animal models (Horton et al., 1973; Garriga-Canut et al., 2006; Stafstrom et al., 2009). 2-DG-induced glucose deprivation is thought to mimic the low glucose availability associated with reduced carbohydrate intake in the KD without a concurrent increase in dietary fats that result in ketosis. Because the seizure protection conferred by antiepileptic drugs in current clinical use does not relate to mechanisms associated with glucose metabolism, these findings are of importance, as they suggest a novel approach to control seizures and could lead to a small molecule replacement for the KD.

The goal of the present study was to further characterize the effects of 2-DG in acute seizure models. Because many anticonvulsant treatments, including the KD (Bough et al., 2000), can show proconvulsant as well as anticonvulsant properties in different seizure tests (Löschter, 2009), and also because severe glucose deprivation can induce seizures, we utilized models that allow proconvulsant as well as anticonvulsant effects to be detected. For comparison, we studied 3-O-methyl-glucose (3-MG), which is transported into cells but is not a substrate for brain hexokinase and, therefore, does not inhibit glycolysis (Colby & Romano, 1975; Jay et al., 1990).

Materials and Methods

Animals

Experimentally naïve male NIH Swiss mice (12–25 g) and male Sprague-Dawley rats (weighing 225–250 g at the beginning of the study) were obtained from the National Cancer Institute (Frederick, MD, U.S.A.) and Taconic Farms (Germantown, NY, U.S.A.), respectively. The animals were housed up to four per cage in a vivarium under controlled laboratory conditions (temperature, 22–26°C; humidity, 40–50%) with an artificial 12-h light/dark cycle.

The animals had free access to a nutritionally balanced rodent chow diet (NIH-07 Rodent Formula Diet) and free access to tap water. Wood chips (Sani-chips, P.J. Murphy Forest Products, Inc., Montville, NJ, U.S.A.) were used in all cages. Animals were allowed to acclimate to the vivarium for at least 3 days before they were used for experiments. The experiments were performed during the light phase of the light/dark cycle between 9:00 and 16:00 h and after a minimum 30-min period of acclimation to the experimental room.

All animals were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and studies were performed under protocols approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke (NINDS) in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Research Council, 1996, National Academy Press, Washington, DC; http://www.nap.edu/readingroom/books/labrats/).

Drugs

The following test compounds were used in the present study: 2-deoxy-D-glucose (2-DG), 3-O-methylglucose (3-MG), pentlyenetetrazol (PTZ), and kainic acid (KA) (Sigma-Aldrich, St. Louis, MO, U.S.A.). All solutions were prepared fresh in sterile 0.9% saline or phosphate buffered saline. 2-DG and 3-MG were administered intraperitoneally (i.p.) in a volume of 0.1 ml/10 and 0.1 ml/100 body weight in mice and rats, respectively. PTZ and KA were administered intravenously (i.v.) in mice. Doses of each compound were expressed in milligram per kilogram.

6-Hz seizure threshold test in mice

The 6-Hz seizure test was performed in accordance with the protocol used by Hartman et al. (2008). Corneal stimulation (0.2 ms square pulse at 6 Hz for 3 s) was administered by a constant-current device (ECT Unit 57800; Ugo Basile, Comerio, Italy). Separate groups of mice were challenged with electrical currents of varying intensities (12–32 mA) to determine the convulsive-threshold strength (CS50) for each dose of drug tested. Ocular anesthetic (0.5% tetracaine hydrochloride ophthalmic solution) was applied to animals’ corneas 30 min before stimulation and 0.9% saline was used to wet electrodes immediately before testing to maintain good electrical contact. Mice were manually restrained during stimulation. Immediately following stimulation, mice were placed in a Plexiglas arena (27.5 cm × 20 cm × 15 cm) for behavioral observation. Animals exhibited seizures characterized by a stunned (fixed) posture, which was often followed by rearing, forelimb clonus, and twitching. At the end of the seizure, animals resumed normal exploratory behavior. Control animals always exhibited more than 10 s of abnormal behavior; treated animals were considered to be protected if the abnormal behavior was <10 s in duration.

Mouse electroshock seizure threshold (MEST) test

Corneal stimulation (60 Hz sine wave for 0.2 s) was administered by a custom made constant-current device. As in the 6-Hz seizure test, ocular anesthetic (0.5% tetracaine hydrochloride ophthalmic solution) was applied to animals’ corneas 30 min before stimulation, and 0.9% sterile saline was used to wet electrodes immediately before testing. Mice were manually restrained during stimulation. To evaluate convulsive-threshold strength (CS50), separate groups of mice were challenged with electrical currents of varying intensities (6–20 mA) and the endpoint of tonic hind limb extension was recorded (Giardina & Gasior, 2009). Each mouse was stimulated only once.

Intravenous PTZ and KA seizure threshold tests in mice

Mice were placed in a Rotating Tail Injector (Braintree Scientific, Braintree, MA, U.S.A.), which is a plastic cylinder (11.25-cm long, 3-cm inner diameter) with a plunger...
for restraint. The lateral tail vein was catheterized with a 1.25-cm long, 30-gauge needle attached to a 25-cm length of polyethylene tubing (PE-10). After correct needle placement into the tail vein was verified by the appearance of blood in the tubing, the needle was secured gently to the tail using plastic tape. The tubing was attached to a 12-ml plastic syringe containing either PTZ or KA solution. The syringe was mounted on an infusion pump (KD Scientific, Holliston, MA, U.S.A.). Following catheterization, mice were released from the restrainer and placed in a Plexiglas arena (27.5 cm x 20 cm x 15 cm) for behavioral observation during the infusion. PTZ (10 mg/ml) and KA (5.33 mg/ml) were infused at a rate of 0.05 and 0.125 ml/min, respectively. These infusion parameters were determined to be optimal for the reliable assessment of seizure threshold.

For PTZ infusions, three signs of seizure activity that typically occurred in sequence were used to determine the threshold for seizure induction: (1) tail twitch (rapid upward flick of rigid tail), (2) clonus (repeated jerking movements of all four limbs lasting at least 5 s) with loss of the righting reflex (clonic seizure), and (3) tonic hindlimb extension (tonic seizure). For KA infusions, the following signs were indications of seizure progression: (1) behavioral arrest (sudden decrease in ambulatory activity during the infusion), (2) clonus (repeated jerking movements of all four limbs) with loss of the righting reflex (clonic seizure), and (3) tonic forelimb or hindlimb extension (tonic seizure). The times between the start of the infusion and the onset of these endpoints were recorded and used to calculate seizure thresholds for each endpoint separately. Seizure threshold was calculated using the following formula: threshold dose [mg/kg] = (drug concentration [mg/ml] x infusion rate [ml/s] x infusion duration [s] x 1,000)/body weight [g]. Seizure threshold was expressed as a dose of PTZ or KA (in mg/kg) needed to produce a given endpoint as noted above. For PTZ, the infusion was stopped at the beginning of the tonic seizure, which was usually lethal; for KA, the infusion was terminated at the beginning of the clonic seizure. Our protocol permitted a maximum infusion time of 10 min, at which point the infusion was terminated if a tonic seizure, which was usually lethal; for KA, the infusion was stopped at the beginning of the clonic seizure. Rats that failed to show the anticipated response to electrical stimulation were eliminated from the study before the start of drug testing.

Rats were stimulated individually within a 29-cm diameter acrylic glass cylinder. Each rat was connected to a custom-made stimulator via a swivel attachment to allow free movement within the chamber. The stimulator was set to deliver 1-ms, bipolar, square current pulses at 60 Hz for 1-s duration at variable current intensities. Depth electroencephalography (EEG) signals were recorded via the stimulating electrode (except during the 1-s stimulation interval) with a Grass CP511 AC EEG preamplifier (Astro-Med, West Warwick, RI, U.S.A.) and stored in digital form using Axotape 9 (Axon Instruments, Foster City, CA, U.S.A.).

Kindled seizure activity was assessed using four measures: afterdischarge (AD) threshold, AD duration, severity of behavioral seizure, and duration of behavioral seizure. AD threshold refers to the lowest stimulating current intensity (in μA) that induces an AD consisting of a train of EEG spikes at 1 Hz or more lasting for at least 5-s duration with amplitude of at least twice the baseline amplitude. AD duration reflected the total duration of the AD (in seconds). The severity of the behavioral seizure was scored according to Racine (1972), with the following designations: stage 0, no apparent change in behavior; stage 1, facial twitching; stage 2, head nodding associated with more severe facial twitching; stage 3, forelimb clonus; stage 4, rearing; stage 5, rearing and loss of balance. Duration of behavioral seizure (in seconds) reflected the duration of the limbic seizure (stage 1–2) or the motor

**Amygdala kindling in rats**

Each rat was chronically implanted with a bipolar stimulating electrode assembly (Plastics One, Roanoke, VA, U.S.A.) during an aseptic surgical procedure under general anesthesia induced by a mixture of ketamine and medetomidine (see Gasior et al., 2007). The electrode assembly consisted of two 0.23 mm-diameter stainless steel electrode wires separated by 0.5 mm. The electrode wires were polyamide-insulated except for the 0.5 mm most distal extent. The electrodes were fixed to a threaded central plastic pedestal with pin connectors for connection to a kindling stimulator. The bipolar electrodes were used for recording and stimulating. The electrode tip was implanted into the basolateral nucleus of the right amygdala at stereotaxic coordinates (AP: –2.8; ML: 5.0; DV: –8.7) measured from bregma (Paxinos & Watson, 1998). Dental acrylic cement (Lang Dental, Wheeling, IL, U.S.A.) and stabilizing stainless steel screws (Plastics One) were used to secure the electrode assembly to the skull. Ketoprofen was given subcutaneously after surgery, followed by atipamezole (1 mg/kg) to reverse anesthesia. At least 10 days were allowed for recovery after the surgery. The position of the electrode assembly tip was histologically verified in randomly selected rats at the end of the study. Rats that failed to show the anticipated response to electrical stimulation were eliminated from the study before the start of drug testing.

Rats were stimulated individually within a 29-cm diameter acrylic glass cylinder. Each rat was connected to a custom-made stimulator via a swivel attachment to allow free movement within the chamber. The stimulator was set to deliver 1-ms, bipolar, square current pulses at 60 Hz for 1-s duration at variable current intensities. Depth electroencephalography (EEG) signals were recorded via the stimulating electrode (except during the 1-s stimulation interval) with a Grass CP511 AC EEG preamplifier (Astro-Med, West Warwick, RI, U.S.A.) and stored in digital form using Axotape 9 (Axon Instruments, Foster City, CA, U.S.A.).

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seizure (stage 3–5). Behavioral changes such as immobility with occasional facial twitches that often occurred after the end of the motor seizure were not considered in the duration determination.

After the rats recovered from the surgery, kindling began and consisted of three phases: (1) prekindling determination of the AD threshold; (2) kindling development; and (3) postkindling redetermination of the AD threshold (Pinel et al., 1976; Freeman & Jarvis, 1981; Gasior et al., 2007). On day 1, AD threshold was determined by delivering a series of stimulations of increasing intensity (starting at 50 μA and increasing in 25% increments every 3–5 min) until an AD was triggered. Rats were excluded from the study if a current of 466 μA intensity failed to produce an AD on the first day of kindling. During the second phase of kindling, each rat was stimulated daily at a current intensity of 125% of its individual AD threshold value determined on day 1. Daily stimulations continued until the rat exhibited stage 5 seizures during five consecutive days or on 8 days out of the last 10 stimulation days. Rats that met this criterion (within, on average, 16 ± 2 stimulations) were considered to be “kindled.” Rats that failed to meet the kindling criterion within 30 stimulations were excluded from the study. During the third phase of kindling, AD threshold was redetermined in the same way as during the first phase of kindling. Threshold was redetermined on several consecutive days until both AD threshold and seizure activity produced by stimulation at the AD threshold were stable and reproducible. Rats that did not show stable responses to the electrical stimulation at their respective AD thresholds for several consecutive days were excluded from further testing.

Once the rats met the kindling criterion, different doses of 2-DG and vehicle were administered in a random order 30 min prior to the kindling stimulation. There were no more than two testing sessions per week and each testing session was separated by at least 2 days to ensure there were no lingering effects on amygdala-kindled seizures from the previous testing.

Determination of plasma glucose and β-hydroxybutyrate levels in mice

Trunk blood glucose and β-hydroxybutyrate levels were measured in selected mice using a Precision Xtra Advanced Diabetes Management System with Precision Xtra blood β-ketone test strips and blood glucose test strips (Abbott Diabetes Care Inc., Alameda, CA, U.S.A.) as described by Schewchter et al. (2003) and Hartman et al. (2008). The test apparatus was calibrated at the start of each experiment. Animals were euthanized in a CO₂-infused plastic chamber. Immediately following euthanasia, animals were decapitated and blood was applied to the test strip. Plasma concentrations of glucose and β-hydroxybutyrate were expressed in milligrams per milliliter and millimolar, respectively.

Plan of experiments

2-DG was first tested in the 6-Hz seizure test, a seizure paradigm found to be sensitive to the anticonvulsant effects of the KD (Hartman et al., 2008; Samala et al., 2008). In the 6-Hz seizure test, variable pretreatment times were tested with a dose of 2-DG (250 mg/kg) that was reported to be effective in several rodent seizure tests (Stafstrom et al., 2009). After the optimal pretreatment time for this dose of 2-DG was determined, dose–response data were obtained at that pretreatment time. Plasma glucose and β-hydroxybutyrate levels were determined at all doses. The effects of 2-DG at 250 and 500 mg/kg were then assessed in the MEST, i.v. PTZ and i.v. KA seizure tests in mice. 2-DG was also tested in fully amygdala-kindled rats. In addition, 3-MG was evaluated in the mouse 6-Hz and i.v. PTZ seizure tests.

Statistical analysis

For mouse studies, each experimental group consisted of at least eight mice. A log-probit analysis was used for statistical determination of CS₅₀ values with 95% confidence limits (95% CLs) in the 6-Hz and MEST seizure tests (Litchfield & Wilcoxon, 1949). Each CS₅₀ value represents an estimate of the median current intensity (in mA) required to induce seizures in 50% of mice tested. Threshold values obtained in the i.v. PTZ and i.v. KA seizure tests were expressed as group means ± standard error of the mean (SEM). Those data were analyzed by means of one-way analysis of variance (ANOVA) followed by Dunnett’s test for specific post hoc comparisons. Likewise, pharmacokinetic data of plasma glucose and β-hydroxybutyrate levels were expressed as group means ± SEM and analyzed by means of one-way ANOVA followed by Dunnett’s test for specific post hoc comparisons. For kindling studies, a within-subject design was used in which the same six rats received vehicle, 250 mg/kg 2-DG or 500 mg/kg 2-DG, in a randomized order. The data in the kindling experiments were analyzed using a two-way repeated-measures ANOVA. Regardless of the statistical test used, differences were considered statistically significant when the statistical probability of error was <0.05 (p < 0.05).

Results

Effects of 2-DG on seizure thresholds in the 6-Hz, MEST, i.v. PTZ, and i.v. KA seizure tests

2-DG (250 mg/kg) was evaluated in the 6-Hz seizure test with pretreatment intervals (time between i.p. injection and administration of the electrical stimulus) of 30, 60, and 120 min (Fig. 1, top). At the 30 min pretreatment time, the mean CS₅₀ value for 6-Hz–induced seizures was significantly elevated from the control value. No significant elevation was obtained with pretreatment intervals of 60 and 120 min. Therefore, further testing was conducted.
using a 30-min pretreatment interval. In a dose–response study with this pretreatment interval, 2-DG at doses of 125–500 mg/kg elevated CS\textsubscript{50} values, whereas a dose of 62.5 mg/kg had no effect (Fig. 1, bottom). Furthermore, at all doses of 2-DG tested (62.5–500 mg/kg), 100% of mice tested passed the inverted screen test, showing no evidence of motor impairment.

In contrast to the results with the 6-Hz seizure test, 2-DG was associated with a reduction in threshold in the MEST test (Fig. 2) and the i.v. PTZ and i.v. KA seizure tests (Fig. 3). Depending on the test and specific endpoint measure, the lowest effective dose was either 250 or 500 mg/kg, and the effect was generally dose-dependent (except for tonic seizure threshold in the i.v. PTZ seizure test, which failed to be affected).

**Effects of 2-DG on amygdala kindled seizures in rats**

In fully kindled rats, administration of 250 or 500 mg/kg 2-DG 30 min prior to stimulation failed to affect the threshold current required to induce an electrographic seizure (AD) as recorded from the stimulating electrode (Fig. 4). Similarly, 2-DG had no effect on the AD duration or the characteristics of evoked behavioral seizures (seizure score or duration).
Effects of 3-MG on seizure threshold in the 6-Hz and i.v. PTZ seizure tests

Unlike 2-DG, 3-MG (250 and 500 mg/kg) had no effect on seizure threshold in the 6-Hz test (Fig. 5). Like 2-DG, however, 3-MG at 250 and 500 mg/kg significantly decreased thresholds in the i.v. PTZ test for induction of tail twitch and clonic seizures but not for tonic seizures.

Effects of 2-DG on plasma glucose and $\beta$-hydroxybutyrate

Treatment with 125 and 250 mg/kg 2-DG caused modest but not significant increases in mean plasma glucose 30 min following administration; the increase was statistically significant following a 500 mg/kg dose (Fig. 6). 2-DG treatment had no effect on plasma $\beta$-hydroxybutyrate levels.

**DISCUSSION**

In this study, we found that the effects of acute treatment with 2-DG on seizure threshold vary depending upon the seizure model. In the 6-Hz test in mice, 2-DG transiently raised the seizure threshold, indicating an anticonvulsant action. In fully amygdala-kindled rats, 2-DG failed to affect the stimulation threshold to induce electrographic seizures or the expression of either electrographic or behavioral seizures. In the MEST, i.v. PTZ, and i.v. KA tests, seizure threshold was reduced. Such threshold tests are used in safety pharmacology to assess the potential of agents to promote convulsions (Porsolt et al., 2002). In particular,
the i.v. PTZ test is widely used for this purpose and is well accepted as the standard preclinical method for determining the proconvulsant activity of drugs (Löschcher, 2009; Giardina & Gasior, 2009). Our observation that 2-DG reduces seizure threshold in the i.v. PTZ test was corroborated by Meldrum and Horton (1973) to induce epileptiform changes (electrographic spikes) in the EEG in normal monkeys and in one case myoclonic seizures. However, it is important to recognize that we and Meldrum and Horton (1973) assessed the effects of acute treatment with 2-DG in animals that had not previously been exposed to the substance; our results do not indicate whether 2-DG administered chronically would result in a persistent reduction in seizure threshold.

Our findings are not inconsistent with two recent reports examining the anticonvulsant activity of 2-DG across seizure tests and epilepsy models (Garriga-Canut et al., 2006; Stafstrom et al., 2009). Like Stafstrom et al., we found that 2-DG conferred acute but short-lasting protection in the 6-Hz seizure test and no overt neurobehavioral effects with doses up to 500 mg/kg. However, Stafstrom et al. reported no effect of 2-DG in the maximal electroshock seizure (MES) and subcutaneous (s.c.) PTZ seizure tests in rats, which utilize the same seizure-inducing stimuli as the MEST and i.v. PTZ tests used in the present study. An important difference between the MES and s.c. PTZ tests and the corresponding tests we used is that in the former tests the intensity of the convulsant stimulus is supramaximal and all-or-none seizure protection is the outcome measure. Consequently, these tests are capable of detecting anticonvulsant but not proconvulsant effects of test substances (see Giardina & Gasior, 2009). In contrast, the threshold tests we used have high sensitivity for detecting proconvulsant activity.

In addition to altering the susceptibility to seizures, 2-DG has been reported to protect against kindling development when administered prior to kindling stimulation (Stafstrom et al., 2009). Such an action on kindling development does not imply an effect on seizures per se and, as noted, we did not find 2-DG to confer seizure protection in fully kindled animals. In immature amygdala kindled rats, marked hypoglycemia (20 mg/dl compared with a control value of 93 mg/dl) has been reported to prolong the AD duration although, as in our experiments, there was no effect on AD threshold (Lee et al., 1988). Therefore, even though 2-DG failed to affect kindled seizures in our experiments, we cannot exclude the possibility that 2-DG might, under certain circumstances, cause an exacerbation of kindled seizures.

How does 2-DG elicit anticonvulsant and proconvulsant actions? 2-DG is avidly taken up into cells by hexose transporters and is phosphorylated by hexokinase to form 2-DG-6-phosphate (2-DG-6-P) (Sols & Crane, 1954; Kimmich & Randles, 1976) (Fig. 7). 2-DG-6-P cannot be further metabolized because it competitively inhibits the next step in glycolysis, which is catalyzed by phosphoglucone isomerase (Wick et al., 1957). 2-DG-6-P, therefore, builds to high intracellular levels. These high levels of 2-DG-6-P have been found to allosterically inhibit hexokinase, thus preventing entry of glucose into the glycolytic pathway (Tower, 1958; Chen & Gueron, 1992). Nevertheless, glucose-6-phosphate can still enter the pentose phosphate pathway to generate NADPH but not ATP as in glycolysis. Therefore, as a result of the antimetabolic actions of its downstream product 2-DG-6-P, 2-DG is a potent inhibitor of glycolysis, which shunts glucose metabolism to the pentose phosphate pathway. 3-MG is also a substrate for hexose transporters. However, unlike 2-DG it cannot be phosphorylated by brain hexokinase to any significant extent (Sols & Crane, 1954; Himsworth, 1968; Jay et al., 1990). Therefore, while both 2-DG and 3-MG competitively reduce glucose uptake into cells, 2-DG is a metabolic inhibitor, whereas 3-MG is not. In our experiments with 2-DG and 3-MG, only 2-DG conferred protection in the 6-Hz model, whereas both glucose analogs reduced seizure threshold in the i.v. PTZ test. Therefore, it is plausible that seizure protection conferred by 2-DG results specifically from inhibition of glucose transport.

**Figure 6.** Effects of 2-DG on trunk blood glucose and β-hydroxybutyrate levels. Trunk blood was recovered 30 min after i.p. administration of vehicle (0) or 125–500 mg/kg 2-DG. Samples were collected after seizure testing. Bars represent mean ± SEM of values from 6–8 animals. *p < 0.05 compared with vehicle. *Epilepsia © ILAE
Support for this hypothesis comes from recent work showing that fructose-1,6-bisphosphate (F-1,6-BP) has anticonvulsant activity in various rat seizure models (Lian et al., 2007). F-1,6-BP increases glucose flux into the pentose phosphate pathway and away from glycolysis (Kelleher et al., 1995), as would occur with 2-DG but not 3-MG. In sum, our results are compatible with the conclusion that the anticonvulsant activity of 2-DG results from a limitation of glycolysis and an increase in flux through the pentose phosphate pathway. How this would result in seizure protection remains to be determined. One possibility is that increased levels of NADPH resulting from enhanced pentose phosphate pathway activity would convert endogenous glutathione to its reduced form, which has been shown to have anticonvulsant activity (Abe et al., 2000; Stringer & Xu, 2008).

Although 2-DG but not 3-MG confers seizure protection in select tests, both 2-DG and 3-MG cause seizure threshold reduction in other tests, which could relate to the well-characterized ability of both sugars to prevent glucose uptake into cells or, alternatively, to other actions unrelated to glucose uptake. Severe hypoglycemia is a common metabolic derangement known in the clinical setting to precipitate seizures (Cryer, 1999). Similarly, in experimental animals, marked acute hypoglycemia lowers the threshold for PTZ (Waltregny et al., 1966; Kaul et al., 1980) and fluorothyl seizures (Kirchner et al., 2006) and can induce focal seizures in cats with an epileptic cortical focus (Gastaut et al., 1968). Marked acute hypoglycemia can also induce generalized seizures in normal cats and rats (Gastaut et al., 1968; Panickar et al., 1998; Kirchner et al., 2006). For example, Kirchner et al. (2006) found that the fluorothyl threshold was reduced with blood glucose levels in the range of 30 mg/dl, whereas generalized behavioral and electrographic seizures were induced at a level of ~20 mg/dl. Therefore, acute glucose deprivation of cells whether by a reduction in glucose availability or inhibition of glucose uptake appears to enhance seizure susceptibility. How this influences circuit excitability remains to be determined.

Our observation that 2-DG has proconvulsant properties naturally raises questions about its utility in epilepsy therapy. In this regard, it is worth noting that several marketed antiepileptic drugs, including sodium channel blockers such as phenytoin and carbamazepine, as well as drug that influence the GABA system, such as tiagabine and vigabatrin, also have proconvulsant properties and can induce seizures under certain circumstances (Marchiani et al., 1995; Mecarelli et al., 1997; Löschcher et al., 1998; Löscher, 2009). Therefore, the fact that 2-DG is proconvulsant does not by itself eliminate that possibility that it could be useful in epilepsy therapy.

Figure 7.
Schematic illustration of differential inhibitory effect of 2-DG and 3-MG on glucose transport and metabolism. 2-DG and 3-MG decrease glucose metabolism by decreasing its transport into the cells (indicated by a dashed arrow for glucose) through a competitive block at the level of glucose transporter (Betz & Gilboe, 1974; Pardridge & Oldendorf, 1975; Vyska et al., 1985). After entering the cell, 2-DG is phosphorylated by hexokinase to form 2-deoxyglucose-6-phosphate (2-DG-6-PO4), which is not a substrate for phosphoglucose isomerase. Unable to undergo further metabolism, 2-DG-6-PO4 accumulates within the cell causing an inhibition of hexokinase and phosphoglucose isomerase. This leads to a reduction of glucose metabolism via the glycolytic pathway and shunting through the pentose phosphate pathway (Sols & Crane, 1954). 3-MG is not substantially phosphorylated in brain and is, therefore, unable to directly inhibit glycolysis or cause shunting (Jay et al., 1990). Double arrowheads indicate inhibition.
The concept of using 2-DG as a treatment for epilepsy originated in the observation that blood glucose is modestly reduced and glycolysis is suppressed in the KD (Garriga-Canut et al., 2006). 2-DG was conceived as a way of mimicking the diet-induced decrease in glycolysis. The KD is active in the 6-Hz test as is 2-DG, indicating that there is some similarity in their actions (Hartman et al., 2008; Sama/ala et al., 2008). Moreover, both 2-DG (this study) and the KD (Bough et al., 2000) also can promote seizures. Clearly, the discovery that 2-DG has anticonvulsant properties provides important insight into the development of a nondietary replacement for the KD. Caution is warranted, however, given the potential of approaches that reduce glucose availability to promote seizures. The present work, in conjunction with the observations of Lian et al. (2007) on F-1,6-BP, suggests that it may be worthwhile to seek approaches that decrease glycolysis and shunt carbohydrate metabolism through the pentose phosphate pathway while avoiding suppression of glucose uptake into cells.

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DISCLOSURE

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors have any associations that affect their ability to present and/or interpret the data presented herein objectively. The authors declare no conflicts of interest.

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