Epilepsy Research (2012) **101**, 246—252

journal homepage: www.elsevier.com/locate/epilepsyres

Behavioral, cognitive, and safety profile of 2-deoxy-2-glucose (2DG) in adult rats

Jeffrey C. Ockuly, James M. Gielissen, Craig V. Levenick, Carly Zeal, Katherine Groble, Kaitlyn Munsey, Thomas P. Sutula, Carl E. Stafstrom*

Department of Neurology, University of Wisconsin, Madison, WI 53705, United States

Received 24 September 2011; received in revised form 16 March 2012; accepted 16 April 2012
Available online 10 May 2012

KEYWORDS
Epilepsy;
Animal models;
Toxicity;
Glycolysis;
2-Deoxyglucose

Summary 2-Deoxy-D-glucose (2DG), a glucose analog that transiently inhibits glycolysis, has anticonvulsant and antiepileptic disease-modifying properties in experimental *in vivo* models of seizures and epilepsy. Here we evaluated the effects of 2DG across the range of doses (50—500 mg/kg i.p.) shown previously to exert anticonvulsant and antiepileptic effects in rats, on spatial learning and memory using the Morris water maze and on exploratory behavior using the open field test. For water maze testing, both acute and chronic protocols were tested. For acute testing, 2DG was injected for 15 min prior to the water maze trial only on testing days. For chronic testing, 2DG was injected daily for 14 days before water maze testing began. Neither protocol altered the latency to platform acquisition or retention of platform location by the probe test. For open field testing, 2DG was given at doses of 50—250 mg/kg 15 or 30 min prior to testing on each testing day. When given 30 min prior to testing, exploratory activity in the open field was transiently and reversibly decreased by 2DG at doses of 250 mg/kg/day but there were no effects on open field activity at 50 mg/kg/day. When given 15 min prior to testing, 2DG decreased exploratory activity in a dose-dependent fashion at both 50 and 250 mg/kg. There were no toxic effects of 2DG at doses of 500 mg/kg/day on body weight or general health. In summary, 2DG is well tolerated at doses associated with anticonvulsant and antiepileptic effects, supporting its potential as an anticonvulsant and antiepileptic agent with a novel mechanism of action.

Introduction

The glucose analog 2-deoxy-D-glucose (2DG), which transiently inhibits glycolysis by blocking isomerization of glucose-6-phosphate to fructose-6-phosphate, suppresses interictal and ictal epileptiform activity in hippocampal slices and protects against seizures evoked by audiogenic stimulation in mice, 6 Hz corneal stimulation in rats

* Corresponding author at: Department of Neurology, University of Wisconsin School of Medicine and Public Health, 1685 Highland Avenue, Madison, WI 53705, United States. Tel.: +1 608 262 2154; fax: +1 608 263 0412.
E-mail address: stafstrom@neurology.wisc.edu (C.E. Stafstrom).

0920-1211/$ — see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.eplepsyres.2012.04.012

(Stafstrom et al., 2009; Gasior et al., 2010), and pilocarpine in rats (Lian et al., 2007). 2DG does not protect against maximal electroshock seizures (Lian et al., 2007; Stafstrom et al., 2009; Gasior et al., 2010), pentylenetetrazole (PTZ)-induced seizures (Lian et al., 2007; Stafstrom et al., 2009; Gasior et al., 2010), or kainate-induced acute seizures in rats (Lian et al., 2007).

In addition to these acute *in vitro* and *in vivo* anticonvulsant actions, 2DG also has chronic *in vivo* antiepileptic action against epilepsy progression evoked by kindling of the perforat path and olfactory bulb (Garriga-Canut et al., 2006; Stafstrom et al., 2009). The chronic antiepileptic action against progressive effects of repeated seizures evoked by kindling can be regarded as a ''disease-modifying'' effect against adverse consequences of seizure-induced plasticity in neural circuitry, which include synaptic reorganization and pathway remodeling, memory and cognitive disturbances, and increased susceptibility to additional seizures. The chronic antiepileptic action against seizure progression and plasticity has been linked to the ability of 2DG to reduce seizure-induced increases in brain derived neurotrophic factor (BDNF) and its receptor trkB, by transcriptional regulation involving neuron-restrictive silencing factor (NRSF), its metabolic sensor C-terminal binding protein (CtBP), and chromatin modification (Garriga-Canut et al., 2006). The mechanisms underlying the acute anticonvulsant actions of 2DG are unknown.

The eventual clinical utility of 2DG (or other metabolic inhibitors) for treatment of epilepsy will require demonstration of safety, tolerability, and absence of toxicity at clinically effective doses. 2DG, albeit at far lower doses, has been used safely in humans for decades as a fluorinated positron emitting tracer for measurement and imaging of regional glucose utilization by positron emission tomography (Wree, 1990). 2DG also has been evaluated as an adjunctive chemotherapeutic agent for certain forms of cancer (Pelicano et al., 2006). Here we report the effects of 2DG in the rat on spatial memory, exploratory activity, and body weight. Our results confirm that 2DG is well tolerated at doses with anticonvulsant and antiepileptic effects.

Methods

All experiments were performed in accordance with NIH animal care guidelines as approved by the University of Wisconsin Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats weighing approximately 400 g were used in these experiments. All animals were exposed to a 12-h light/dark cycle, received standard rat chow and water *ad libitum*, and were housed individually in plastic cages.

Effects of 2DG on body weight and blood beta-hydroxybutyrate levels

To assess the effect of 2DG administration on body weight, age-matched 5-month-old rats on a standard rat chow diet were randomized to receive daily intraperitoneal (i.p.) injections of either saline or 2DG 250 mg/kg twice daily (500 mg/kg/day) for 6 months.

Rats were euthanized by CO_2 inhalation at the conclusion of the body weight experiment (age 11 months). Blood beta-hydroxybutyrate (BHB) levels were measured using a Statsite Ketone Monitor (Stanbio Laboratory, Boerne, TX) on blood obtained by vena cava puncture immediately after death.

Water maze

To evaluate the effect of 2DG on learning (acquisition) and memory (retention), a separate group of rats was subjected to spatial learning testing using a Morris water maze (Morris et al., 1982; Stafstrom et al., 1993). A circular steel tank (117-cm diameter) was filled with water ($26 \pm 1\,^{\circ}C$) to a depth of 25 cm. The water was made opaque by addition of ~100 ml evaporated milk. The room was illuminated by overhead lights, and visual cues around the room (furniture, lighting, experimenter position) were kept constant from day to day. Four points on the perimeter of the pool were designated north (N), east (E), south (S) and west (W), dividing the pool into four virtual quadrants (NW, NE, SE, SW). An 8 cm × 8 cm plexiglass platform onto which the rat could escape was positioned in the center of one of the quadrants, 1 cm below the water surface. The opaque water precluded visual determination of platform position, forcing the rats to use distant visual cues to locate it.

On day 1 of training, each rat was placed in the pool for 60 s without the platform present, for habituation to the training environment. On days 2—5, rats were trained for 16 trials (4 trials per day) to locate and escape onto the submerged platform. The quadrant in which the platform was located remained constant, but the point of immersion into the pool varied between N, E, S, and W over the 16 trials so that the rat would not be able to predict the platform location from the starting location. The latency from immersion into the pool to escape onto the platform was recorded for each trial by one observer, while another observer mapped the route taken by the rat to reach the platform. Upon mounting the platform, the rat was given a 30-s rest, after which the next trial commenced. If the rat did not find the platform in 120 s, it was placed on the platform for a 30-s rest.

Four hours after the final training trial on day 5, the spatial bias in the search pattern was tested to assess memory for platform location (''probe trial''). The platform was removed from the pool and the rat was placed back into the pool in the quadrant opposite the previous platform location. During 60 s of free swimming, the rat's swim route was recorded. The distance swum in each quadrant was calculated by tracing the swim path using a digital curvimeter. Swim distances are proportional to the time spent in each quadrant, since swimming speed was equivalent in each group. The number of virtual crossings over the previous platform location was also recorded and compared between groups.

Open field

To assess exploratory activity and locomotion in a novel environment, rats were tested in an open field, consisting of a 45 in. × 45 in. tiled floor area divided into 25 equal-sized squares. The area was enclosed by a 3-foot high opaque

plexiglass wall. The floor and walls were cleaned and deodorized with 70% alcohol and water between each trial. Ambient lighting and environmental conditions, as well as position of observers were kept constant for all trials. At the beginning of each trial, the rat was placed in the same corner square facing the wall. Rat movement was tracked manually for one 5 min-trial daily for 4 consecutive days. The number of line crossings and rearings were recorded. A line crossing was defined as movement of both forepaws across a line.

Statistical analysis

Data for body weights and BHB levels were analyzed using analysis of variance (ANOVA) with *post hoc* Tukey tests. Water maze data were analyzed using 2-way ANOVA with repeated measures for the platform latency test and 2-way ANOVA for probe test measures. Open field data were analyzed by ANOVA with repeated measures with *post hoc* Tukey tests or pairwise multiple comparison procedures (Holm—Sidak method). Significance was defined as $p < 0.05$ for all tests.

Results

Effects of 2DG on body weight and blood beta-hydroxybutyrate levels

To identify potential systemic toxicity of 2DG at higher doses, separate groups of rats were treated with equivalent volumes of saline or 2DG 500 mg/kg/day (which is ~2—10 times greater than doses required for *in vivo* anticonvulsant and antiepileptic effects). Starting at age 5 months and continuing for the next 6 months, rats receiving 2DG at a dose of 250 mg/kg i.p. twice daily (5 days/week) had no differences in body weight compared to age-matched controls ($p = 0.213$, Tukey test). No systematic abnormalities of behavior or general health were noted during routine laboratory handling of any group of rats.

After euthanasia at age 11 months, blood BHB levels were determined and compared between saline controls and rats that received 2DG 500 mg/kg/day for 6 months. There was no significant difference between controls and 2DG-treated animals in BHB levels (in mM): saline controls 0.39 ± 0.03, 2DG 500 mg/kg/day, 0.28 ± 0.04 (t-test, $p = 0.054$, $n = 7$ per group).

Effects of 2DG on hippocampus-based spatial learning and memory

To evaluate the effects of 2DG on hippocampal-dependent spatial learning and memory, two experiments were performed. First, the acute effects of 2DG on learning and memory were determined by administration of 2DG 15 min prior to each water maze training session. Second, we assessed the effects of 2 weeks of daily administration of 2DG prior to learning and memory testing.

In the first experiment, saline (controls, $n = 6$) or 2DG at either of two doses (50 mg/kg or 250 mg/kg i.p. $n = 8$ each) were given 15 min prior to water maze on each day of testing. This time window allowed assessment of a 2DG effect on spatial learning at a time that drug effect is expected to be maximal. There was no effect of either 2DG dose on spatial learning ($F = 1.88$, $p = 0.158$) or dose × day interaction ($F = 0.42$, $p = 0.864$), but there was an effect of testing day ($F = 6.548$, $p < 0.001$), as rats learned the platform location readily over the 4 testing days (Fig. 1A). On the probe test, the percent time spent swimming by each 2DG-treated group was not different from controls (Fig. 1B). The percent time spent swimming in the target quadrant was: controls, 38.2 ± 1.2; 50 mg/kg group, 33.5 ± 3.0; 250 mg/kg group, 36.9 ± 2.6; $F = 0.851$, $p = 0.440$. Likewise, the number of crossings over the previous platform location did not significantly differ between groups: controls, 4.5 ± 0.67; 2DG 50 mg/kg group, 3.1 ± 0.5; and 2DG 250 mg/kg group, 4.1 ± 0.86 ($F = 0.973$, $p = 0.393$).

In the second experiment, separate groups of rats receiving saline or 2DG at doses of 500 mg/kg/day or 1000 mg/kg/day were evaluated in the Morris water maze. Control animals ($n = 6$) received saline twice daily for 14 consecutive days prior to water maze testing. The two experimental groups received 2DG 500 mg/kg/day (250 mg/kg i.p. twice daily, at ~10 AM and ~3 PM, $n = 8$) or 1000 mg/kg/day (500 mg/kg i.p. twice daily, at ~10 AM and ~3 PM, $n = 8$) over the same time period. Water maze testing commenced on the day following the last dose of 2DG. On the first day of testing, the escape latency to find the platform location was longer in both 2DG dose groups compared to controls (~77 s for the two 2DG dose groups *vs.* ~45 s for the controls, $F = 4.21$, $p = 0.031$) but there was no difference between the two 2DG groups. All groups learned to find the platform over the 4 days of testing. In all groups, the latency to find the platform was significantly shorter on day 4 than on day 1 ($p < 0.001$) (Fig. 1C). Overall, there were no significant differences in the effect of treatment (controls *vs.* 2DG-treated groups) on escape latency ($p = 0.238$), and there was no treatment × day effect ($p = 0.074$). Similarly, there were no differences in memory for platform location (probe test) between controls and 2DG-treated rats on two separate measures. The percent time spent swimming in the previous platform quadrant was not significantly different between groups: controls, 38.3 ± 2.0; 2DG 500 mg/kg/day group, 36.0 ± 2.6; and 2DG 1000 mg/kg/day group, 31.4 ± 1.7 ($F = 2.88$, $p = 0.081$) (Fig. 1D). The number of crossings over the previous platform location was also not significantly different between groups: controls, 5.7 ± 1.2; 2DG 500 mg/kg/day group, 4.5 ± 0.5; and 2DG 1000 mg/kg/day group, 5.3 ± 0.7 ($p = 0.56$).

Effects of 2DG on open field activity

To evaluate the effects of 2DG on exploratory behavior, two experiments were performed. First, 2DG was given 15 min prior to each testing session to determine its acute effects on exploratory behavior. Second, the effects of 2DG administration 30 min after administration were assessed using a cross-over design.

In the first open field experiment, rats received saline ($n = 6$), 2DG 50 mg/kg ($n = 10$), or 2DG 250 mg/kg ($n = 10$) i.p. 15 min prior to testing. There was a marked, dose-dependent effect of 2DG on open field behavior as assessed by number

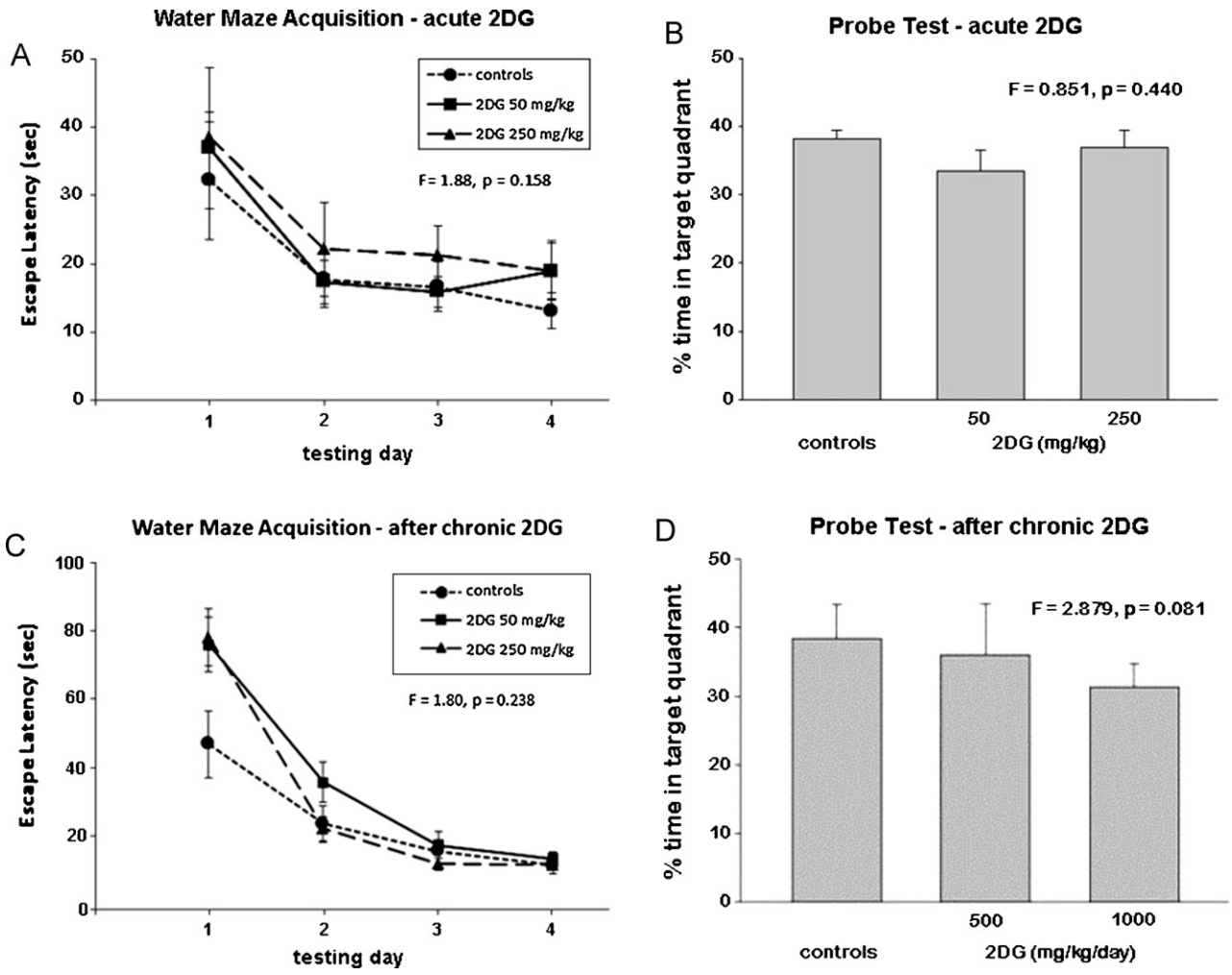

Fig. 1 Water maze. (A) Water maze acquisition learning in rats administered saline, 50 mg/kg 2DG, or 250 mg/kg 2DG 15 min prior to testing on each of 4 testing days. There was no difference in platform acquisition among groups. (B) Probe test comparing control and 2DG-treated rats with saline or 2DG administered 15 min prior to testing each day. There was no difference in the time spent by any group in the target quadrant. (C) Comparison of control and 2DG-treated rats on water maze acquisition learning. Controls were treated with twice daily saline injections for 2 weeks prior to behavioral testing; experimental animals were treated with twice daily injections of 2DG at 500 or 1000 mg/kg/day for 2 weeks prior to behavioral testing. There was no difference between groups in learning the position of the platform over the 4 days of testing. (D) Comparison of control and 2DG-treated rats on the water maze probe test. There was no difference in the time spent by any group in the target quadrant. Error bars are mean ± S.E.M.

of lines crossed ($F = 53.4$, $p < 0.001$) (Fig. 2A) but no difference by day of testing ($F = 1.738$, $p = 0.165$) or dose × day interaction ($F = 0.653$, $p = 0.688$). The number of rearings also differed significantly by dose group (Fig. 2B), with fewer rearings as a function of 2DG dose ($F = 40.1$, $p < 0.001$) as well as testing day ($F = 8.20$, $p < 0.001$) and dose × day ($F = 2.85$, $p = 0.014$).

In the second experiment, 36 3-month-old rats were divided into 3 groups ($n = 12$ each) and compared in a cross-over design. One group received 2DG 50 mg/kg i.p. 30 min prior to open field testing. A second group received 2DG 250 mg/kg i.p.30 min prior to testing. The control group received a comparable volume of saline. Each rat was placed in the open field and activity was evaluated for 4 days as described in Methods. After a 3-day wash-out period with no drug treatment, the rats initially treated with saline were switched to 2DG at a dose of 250 mg/kg i.p. and rats that were initially treated with 2DG 250 mg/kg i.p. received saline injections for the next 4 days. The group that initially received 2DG 50 mg/kg i.p. continued to receive 50 mg/kg i.p. for the second 4-day set of trials. There was no difference in number of lines crossed or number of rearings between the saline and 2DG 50 mg/kg groups, but rats receiving 2DG 250 mg/kg crossed significantly fewer lines ($F = 20.1$, $p < 0.001$) (Fig. 2C). Rats initially treated with saline and then switched to 2DG 250 mg/kg (cross-over), had reduced open field activity compared to the saline-treatment period prior to cross-over and to both the saline-controls and rats treated with 2DG at a dose of 50 mg/kg i.p. (Fig. 2C, after cross-over). After the 3-day washout, saline-treated rats (previously treated with 2DG 250 mg/kg) had increased open field activity, comparable to the initial saline-treated controls and the group treated with 2DG 50 mg/kg. Rearings, another measure of open field

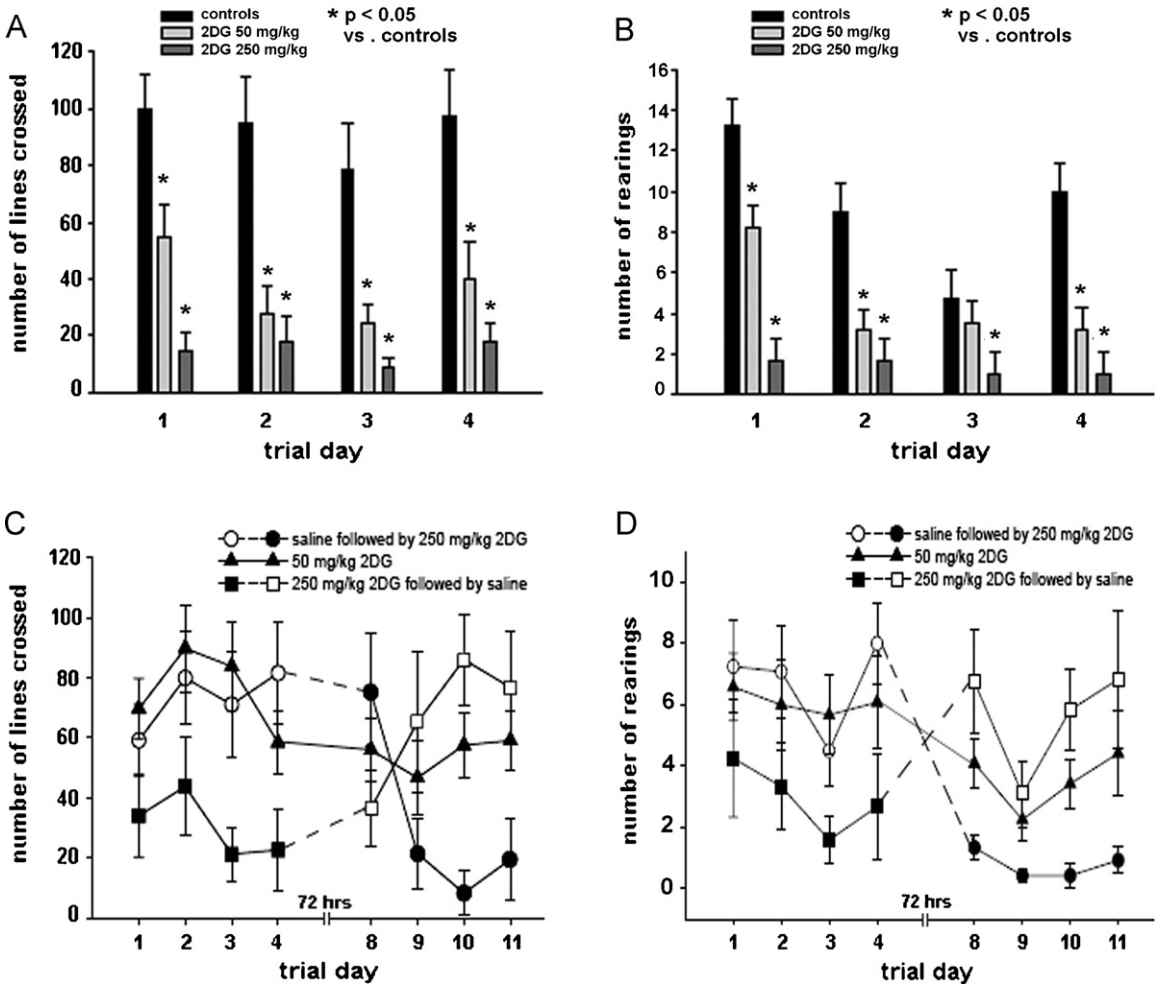

Fig. 2 Open field testing in saline controls and two 2DG dose groups (50 mg/kg and 250 mg/kg). (A and B) Acute protocol. 2DG was administered 15 min prior to open field testing each day. (A) Number of line crossings. (B) Number of rearings. The 250 mg/kg 2DG dose was associated with a reversible decrease in open field locomotor activity (both number of lines crossed and number of rearings). Single and double asterisks indicate significant difference from controls at $p = 0.05$ level for each dose group, respectively. (C and D) Cross-over design. 2DG was administered 30 min prior to open field testing each day over 4 days, followed by a 3-day washout period, followed by a second 4-day testing period in which the saline and 250 mg/kg groups crossed over. (C) Number of line crossings. (D) Number of rearings. For both line crossings and rearings, rats receiving 2DG 250 mg/kg had reversible reduction on locomotor activity. Error bars are mean ± S.E.M. See text for details.

activity, were similarly reduced in the rats during periods of treatment with a dose of 250 mg/kg i.p. compared to the saline-treated controls and the group that received 2DG 50 mg/kg ($F = 23.6$, $p < 0.001$) (Fig. 2D). The reduction of open field activity during treatment periods of 250 mg/kg i.p. in this cross-over study demonstrates that the effects of 2DG at this dose are reversible.

Discussion

The main findings of this study are that treatment of rats with 2DG at doses of up to 1000 mg/kg/day i.p. for 14 days had no apparent detrimental effect on spatial learning and memory as assessed by the water maze, but doses of 250 mg/kg i.p. were associated with a reversible decline in exploratory behavior as assessed by open field measures (number of lines crossed, number of rearings). As ED50 for 2DG in the *in vivo* models of 6 Hz stimulation in mice, audiogenic seizures in Fring's mice, and kindling in rats ranged from 50 to 206 mg/kg (Stafstrom et al., 2009), these results suggest that treatment with 2DG in dosage ranges associated with anticonvulsant and antiepileptic effects is well-tolerated, specifically in regard to hippocampus-dependent spatial memory. Chronic doses of 500 mg/kg/day i.p. for 6 months were well tolerated in terms of maintenance of weight and systemic health. This study did not include formal pathological examination, which will be pursued in future studies.

This is the first report of the effects of 2DG, an inhibitor of glycolysis, on rat spatial learning and memory. At doses effective in decreasing seizures and epilepsy progression, 2DG had no deleterious effects on rat performance in the water maze, considered to be a measure of

hippocampus-dependent spatial cognition (Morris et al., 1982). These results with 2DG differ from effects of dietary manipulations such as calorie restriction, which would also be anticipated to modify carbohydrate metabolism; in contrast, calorie restriction has been shown to increase motor learning and consolidation in mice, as well as enhance synaptic efficacy, possibly related to increased hippocampal expression of NMDA receptor subunit NR2B (Fontan-Lozano et al., 2007).

In the open field experiments, 2DG, when administered 15 min prior to testing, reduced exploratory activity in a dose-dependent manner, with the effect most marked with the 250 mg/kg dose. Open field activity was not reduced with 50 mg/kg 2DG when administered 30 min prior to testing, suggesting an effect of time of administration which will be addressed in future pharmacokinetic studies. In the crossover experiment, in which 2DG was administered 30 min prior to open field testing, the 250 mg/kg dose, but not the 50 mg/kg dose, was associated with decreased movement in the test arena and reduced numbers of rearings. When rats exposed to 2DG 250 mg/kg were crossed over to saline injections, their activity level increased to that seen in saline controls. Similarly, when control rats were crossed over to the 2DG 250 mg/kg dose, their open field activity decreased. Therefore, reduction of open field activity is a reversible effect of 2DG. Interestingly, rats placed on a ketogenic diet also displayed a reversible decrease in open field activity, independent of anxiety (Murphy and Burnham, 2006). These results raise the possibility that glucose restriction decreases some aspect of motor activity. On routine observation, we did not notice differences in baseline motor activity of any experimental group.

Our study confirms other reports indicating that 2DG has a favorable safety profile in the range of doses associated with anticonvulsant effects, but other studies have shown that 2DG has adverse cardiac effects at higher doses. Oral or intravenous doses of 2DG 250—2000 mg/kg given to rats or mice over 7 days a caused dose-dependent fall in mean arterial pressure and respiratory rate and increased mortality (Vijayaraghavan et al., 2006). A recent detailed pathological evaluation of chronic oral 2DG ingestion in two rat strains reported cardiotoxic effects with vacuolization of cardiac myocytes, increased incidence of pheochromacytomas, and reduced lifespans (Minor et al., 2010). The cardiac vacuolization in 2DG-treated rats in this study had features consistent with autophagy (Hotchkiss et al., 2009), evidenced by higher expression of cathepsin D and LC3. While it is difficult to compare oral and parenteral 2DG doses, as well as interspecies differences, further studies are needed to assess reversibility of histological effects observed with 2DG treatment.

Since as many as one-third of patients with epilepsy are refractory to standard anticonvulsants, alternative treatments are necessary. Dietary treatments of epilepsy, such as the ketogenic diet and its variants, have received increasing attention in recent years (Stafstrom, 2004). Restriction of calories or carbohydrate intake may be critical for the action of the ketogenic diet (Greene et al., 2001). 2DG or other blockers of glycolysis represent an intriguing, novel approach to seizure suppression (Lian et al., 2007; Stafstrom et al., 2008; Stringer and Xu, 2008). The mechanism of 2DG action could involve altered transcriptional regulation of metabolism (Garriga-Canut et al., 2006), improved mitochondrial function secondary to increased ketogenesis (Yao et al., 2011), or a variety of other mechanisms (discussed in Stafstrom et al., 2008). Of note, 2DG was not associated with altered BHB levels in these experiments.

In summary, our results provide evidence that the glucose analog 2DG at doses associated with anticonvulsant and antiepileptic actions has no significant or permanent adverse effects on hippocampal-based learning and memory or open field activity. These findings, in conjunction with previous reports of 2DG's safety and tolerability in human cancer treatment (Aft et al., 2002; Maschek et al., 2004; Pelicano et al., 2006; Stein et al., 2010) and in rodent models of neuroprotection (Lee et al., 1999; Rejdak et al., 2001), support the potential of 2DG as a safe anticonvulsant and antiepileptic compound with a novel mechanism of action.

References

Aft, R.L., Zhang, F.W., Gius, D., 2002. Evaluation of 2-deoxy-D-glucose as a chemotherapeutic agent: mechanism of cell death. Brit. J. Cancer 87, 805—812.

Fontan-Lozano, A., Sáez-Cassanelli, J.L., Inda, M.C., de los Santos-Arteaga, M., Sierra-Domínguez, S.A., López-Lluch, G., Delgado-García, J.M., Carrión, A.M., 2007. Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. J. Neurosci. 27, 10185—10195.

Garriga-Canut, M., Schoenike, B., Qazi, R., Bergendahl, K., Daley, T.J., Pfender, R.M., Morrison, J.F., Ockuly, J., Stafstrom, C., Sutula, T., Roopra, A., 2006. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. Nat. Neurosci. 9, 1382—1387.

Gasior, M., Yankura, J., Hartman, A.L., French, A., Rogawski, M.A., 2010. Anticonvulsant and proconvulsant actions of 2-deoxy-D-glucose. Epilepsia 5, 1385—1394.

Greene, A.E., Todorova, M.T., McGowan, R., Seyfried, T.N., 2001. Caloric restriction inhibits seizure susceptibility in epileptic EL mice by reducing blood glucose. Epilepsia 42, 1371—1378.

Hotchkiss, R.S., Strasser, A., McDunn, J.E., Swanson, P.E., 2009. Cell death. N. Engl. J. Med. 361, 1570—1583.

Lee, J., Bruce-Keller, A.J., Kruman, Y., Chan, S.L., Mattson, M.P., 1999. 2-Deoxy-D-glucose protects hippocampal neurons against excitotoxic and oxidative injury: evidence for the involvement of stress proteins. J. Neurosci. Res. 57, 48—61.

Lian, X.-Y., Khan, F.A., Stringer, J.L., 2007. Fructose-1,6-biphosphate has anticonvulsant activity in models of acute seizures in adult rats. J. Neurosci. 27, 12007—12011.

Maschek, G., Savaraj, N., Priebe, W., Braunschweiger, P., Hamilton, K., Tidmarsh, G.F., De Young, L.R., Lampidis, T.J., 2004. 2-Deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers *in vivo*. Cancer Res. 64, 31—34.

Minor, R.K., Smith Jr., D.L., Sossong, A.M., Kaushik, S., Poosala, S., Spangler, E.L., Roth, G.S., Lane, M., Allison, D.B., de Cabo, R., Ingram, D.K., Mattison, J.A., 2010. Chronic ingestion of 2-deoxy-D-glucose induces cardiac vacuolization and increases mortality in rats. Toxicol. Appl. Pharmacol. 243, 332—339.

Morris, R.G.M., Garrud, P., Rawlins, J.N., O'Keefe, J., 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681—683.

Murphy, P., Burnham, W.M., 2006. The ketogenic diet causes a reversible decrease in activity level in Long-Evans rats. Exp. Neurol. 201, 84—89.

Pelicano, H., Martin, D.S., Xu, R.-H., Huang, P., 2006. Glycolysis inhibition for anticancer treatment. Oncogene 25, 4633—4646.

Rejdak, K., Rejdak, R., Sieklucka-Dziuba, M., Stelmasiak, Z., Grieb, P., 2001. 2-Deoxyglucose enhances epileptic tolerance evoked by transient incomplete brain ischemia in mice. Epilepsy Res. 43, 271—278.

Stafstrom, C.E., 2004. Dietary approaches to epilepsy treatment: old and new options on the menu. Epilepsy Curr. 4, 215—222.

Stafstrom, C.E., Chronopoulos, A., Thurber, S., Thompson, J.L., Holmes, G.L., 1993. Age-dependent cognitive and behavioral deficits after kainic acid seizures. Epilepsia 34, 420—435.

Stafstrom, C.E., Ockuly, J.C., Murphee, L., Valley, M.T., Roopra, A., Sutula, T.P., 2009. Anticonvulsant and antiepileptic actions of 2-deoxy-D-glucose in epilepsy models. Ann. Neurol. 65, 435—447.

Stafstrom, C.E., Roopra, A., Sutula, T.P., 2008. Seizure suppression via glycolysis inhibition with 2-deoxy-D-glucose. Epilepsia 49 (Suppl. 8), 97—100.

Stein, M., Lin, H., Jeyamohan, C., Dvorzhinski, D., Gounder, M., Bray, K., Eddy, S., Goodin, S., White, E., Dipaola, R.S., 2010. Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. Prostate 70, 1388—1394.

Stringer, J.L., Xu, K., 2008. Possible mechanisms for the anticonvulsant activity of fructose-1,6-diphosphate. Epilepsia 49 (Suppl. 8), 101—103.

Vijayaraghavan, R., Kumar, D., Dube, S.N., Singh, R., Pandey, K.S., Bag, B.C., Kaushik, M.P., Sekhar, K., Dwarakanath, B.S., Ravindranath, T., 2006. Acute toxicity and cardio-respiratory effects of 2-deoxy-2-glucose: a promising radio sensitiser. Biomed. Environ. Sci. 19, 96—103.

Wree, A., 1990. Principles of the 2-deoxyglucose method for the determination of the local cerebral glucose utilization. Eur. J. Morphol. 28, 132—138.

Yao, J., Chen, S., Mao, Z., Cadenas, E., Brinton, R.D., 2011. 2-Deoxy-D-glucose treatment induces ketogenesis, sustains mitochondrial function, and reduces pathology in female mouse model of Alzheimer's disease. PLoS One 6, e21788.