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Oral administration of fructose-1,6-diphosphate has anticonvulsant activity

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Abstract

Recently it has been shown that fructose-1,6-diphosphate (FDP) has dose-dependent anticonvulsant activity in rat models of acute generalized motor seizures induced with chemical convulsants. This present study asked whether FDP also has activity in an epileptic brain after oral administration and activity against non-convulsive seizures. Animals (n=14) were administered pilocarpine to induce status epilepticus. Several weeks later, these animals had spontaneous seizures and a baseline rate of seizure frequency was determined over a 6 day period. Animals were then continued without treatment (n=8) or 0.5% FDP was added to the drinking water (n=6). In animals treated with FDP the seizures completely stopped after 7 days. Removal of FDP from the water resulted in the return of seizure activity in 4 of the 6 animals by 16 days of observation. To induce non-convulsive seizures, animals (n=6) received a single injection of γ -butyrolactone (GBL, 100 mg/kg ip). All animals had spike-wave activity recorded in the cortex within minutes after GBL administration. Administration of a single injection of FDP (500 g/kg ip) had no effect on the baseline cortical activity, nor on the spike-wave activity induced by GBL (n=5). These experiments suggest that oral administration of FDP may have utility in the treatment of partial or generalized convulsive seizure disorders, but not absence seizures.

Keywords

epilepsy; pilocarpine; seizures; gamma-butyrolactone

Introduction

Recently it has been shown that fructose-1,6-diphosphate (FDP, also called fructose-1,6-bisphosphate) has dose-dependent anticonvulsant activity in rat models of acute seizures induced by pilocarpine, kainic acid, or pentylenetetrazole when given intraperitoneally [8]. Each of these models induces generalized motor convulsions. Whether FDP has efficacy in models of absence, or non-convulsive, seizures has not been tested. In addition, the efficacy of FDP in epilepsy, the chronic condition with spontaneous seizures, has not been tested. This study tested whether FDP has anticonvulsant activity in an acute model of non-convulsive seizures and in a model of epilepsy where spontaneous seizures occur weeks after pilocarpine-induced status epilepticus.

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There is debate about whether phosphorylated sugars can cross membranes. However, we have recently demonstrated a significant increase in levels of FDP in the brain after both intraperitoneal and oral administration [14]. Based on this data, we used oral administration of FDP for testing the efficacy in the pilocarpine model of epilepsy where the seizures are quite infrequent.

Materials and Methods

All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH publication 8023, revised 1996) and with the approval of the local Animal Use Committee. Unless indicated, all chemicals were obtained from Sigma Chemical Co (St. Louis, MO). The FDP utilized in these experiments was the dicalcium salt. Male, Sprague Dawley rats weighing 125-250 g (n=25) were used in this study.

For the generation of spontaneous seizures, acute status epilepticus was induced by pilocarpine (subcutaneous scopolamine methyl bromide 1 mg/kg followed 15 min later by intraperitoneal 300 mg/kg, pilocarpine) in 14 adult rats. Behavioral seizures were scored according to an adjusted version of the scale of Racine [3]: stage 1, trembling; stage 2, head bobbing and stereotypies; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus; stage 5, rearing and falling; stage 6, jumping and/or running followed by falling. Animals who had experienced forelimb clonus (stage 4) or rearing and falling (stage 5) for at least 2 hours were defined as having had status epilepticus and kept for the current experiments.

Beginning one week after status epilepticus, the animals were monitored daily from 8 am to 6 pm by an investigator blinded to the treatment group. All behavioral seizure activity was recorded. Ten to 30 days after the injection of pilocarpine, animals showed a variety of seizure behaviors, including facial clonus, isolated jerking, forelimb clonus and forelimb clonus with rearing and falling. Once an animal had experienced at least one spontaneous seizure in 3 out of 7 consecutive days, a baseline value for seizure frequency was established over a 6 day period. Any episode of forelimb clonus with or without rearing was counted as a seizure. Thus, the frequency of seizures scored $\geq$ stage 3 was determined for each animal.

After obtaining the baseline seizure frequency for a minimum of 6 days, animals were assigned to continue drinking normal water (n=8, as controls) or water containing 0.5% FDP (n=6) for 12-14 days (along with normal rat chow). All seizure activity was noted during the treatment period and for up to 16 days after the treatment was stopped. As previously described [5,9], these animals have very few seizures and the seizure frequency can vary considerably across animals in the same experimental group. Therefore, the baseline value for seizure frequency in each animal was normalized to 100%. The number of seizures recorded each day after randomization was then normalized to the baseline value. The percent of baseline seizure frequency for each treatment day was averaged across animals in that treatment group. Even with this normalization the variability in the groups was quite large. In the control group, on any particular day at least 1 animal did not have a behavioral seizure. The normalized data was compared with a 2-way ANOVA comparing the effect of drug treatment and time. A difference was considered significant if the calculated p value was < 0.05 .

Intraperitoneal administration of γ -butyrolactone (GBL) was used as a model of absence seizures [10]. GBL is an inactive prodrug for γ -hydroxybutyrate (GHB). Administration of GBL, instead of GHB, shortens the time to onset and improves the reproducibility of the spike-wave discharges [11]. Since the seizures in this model are non-convulsive, seizure activity was documented by recording electrical activity in the cortex before and after administration of the GBL. Adult male Sprague-Dawley rats (125 - 250g, n=11) were anesthetized with ketamine (25 mg/kg), xylazine (5 mg/kg) and acepromazine (0.8 mg/kg) i.p. and placed into a stereotaxic

frame. Body temperature was maintained at $37 \pm 0.1^\circ\text{C}$ with a water-recirculating heating pad. Three burr holes were drilled into the skull. One was placed on the left side approximately 1.0 mm lateral and 3.0 mm posterior to bregma. A Teflon-coated stainless steel wire was placed into this hole and lowered to 1.0 mm below the dura for recording electrical activity in the cortex. The second burr hole was placed on the right side, 4 mm anterior to bregma. The ground screw and wire were placed in this hole. The final burr hole was placed posterior to the recording electrode and a skull screw was placed into this hole to anchor the dental cement, which was used to fix the assembly to the skull. The animals were maintained on the heating pad until they had fully recovered from the anesthesia.

The electrical recordings were initiated after the animals had completely recovered from anesthesia. After obtaining baseline electrical recordings, the animals were divided into two groups. The first group (n=6) received a single intraperitoneal dose of 100 mg/kg GBL. The other group (n=5) received an injection of FDP (500 mg/kg ip) followed 1 hour later by 100 mg/kg GBL. This dose and timing was chosen based on kinetic data demonstrating maximal increases in brain levels of FDP 1 hour after ip administration of 500 mg/kg [14]. In all animals, the electrical activity in the cortex was continuously monitored and recorded for at least 1 hour after administration of the GBL.

Results

Efficacy of oral administration of FDP on spontaneous seizures

Injection of pilocarpine induced status epilepticus in all animals. Ten to 30 days after the injection of pilocarpine, animals began to have behavioral seizure activity. The mean latency to the first episode of forelimb clonus was 21 ± 3 days (n=14), which is consistent with previous reports [5]. After determination of the baseline frequency of $\geq$ stage 3 seizures, animals were assigned to continue to receive normal tap water or to receive tap water with the addition of 0.5% FDP. All animals were monitored daily for at least 12 days (Figure 1). Control animals (n=8) had no significant change in the seizure frequency. The animals receiving 0.5% FDP in the drinking water (n=6) had a gradual decrease in number of seizures each day until the seizures stopped after 2, 4, 5 (2 animals), 6 or 7 days of treatment. There was no change in the character or type of seizure observed, just a decline in the number. None of the animals in the treatment group had seizures after 7 days.

Animals received FDP for 12-14 days and then they were switched back to tap water and monitored for an additional 16 days. After stopping the FDP, no seizures were observed in the treatment group for the first 10 days. Over the remaining 6 days of the observation period, 4 animals (out of 6) had recurrence of their seizures (2 on day 11, 1 on day 14 and 1 on day 15). The seizures that appeared were indistinguishable from the seizures in the pre-treatment period. No seizures were noted in the remaining 2 animals within the observation period.

Efficacy of FDP on spike-wave discharges induced with GBL

After administration of GBL, spike-wave activity appeared in the cortex within the first 3 minutes in all animals (n=6, mean 128 sec, range 118-146 sec), which is consistent with previous reports [2,10,11]. With the appearance of the spike-wave activity in the cortex the animal stopped most spontaneous movement, but no motor seizure activity was ever observed. The spike wave activity lasted 30 minutes with a gradual decrease in amplitude and frequency. Therefore, animals were monitored continuously for 1 hour after administration of GBL. Pretreatment with FDP, at 500 mg/kg (n=5), had no effect on baseline electrical activity and did not alter the spike-wave activity that appeared after administration of GBL. There was no change in the latency to onset of the spike-wave activity (mean 128 sec, range 119 - 137 sec), the frequency of the activity at 15 minutes (mean 4 spikes per second for both groups) or the

duration of the activity (range of 15-20 minutes for both groups) compared to animals not treated with FDP.

Discussion

Previous experiments have demonstrated that acute administration of FDP has anticonvulsant activity in rat models of acute seizures [8]. The present study indicates that FDP has activity against spontaneous seizures in a model of epilepsy generated by pilocarpine-induced status epilepticus and has activity when administered orally. FDP did not have an effect in the model of non-convulsive seizures at the dose (500 mg/kg) which was effective against convulsive seizures [8]. While clinical testing will be necessary, these data suggest that orally administered FDP may have activity against partial seizures and generalized tonic-clonic seizures, but not absence seizures.

There are a number of possible mechanisms for the actions of FDP and theories about mechanisms underlying the different seizure types that might help interpret the different efficacy of FDP in the different models. FDP has been shown to shift the metabolism of glucose from the glycolytic pathway to the pentose phosphate pathway [6,7]. Diverting glucose towards the pentose phosphate pathway should increase the formation of glutathione, an endogenous anticonvulsant [1,13], but not decrease the availability of energy. Reduction of glycolytic activity might be predicted to be more effective against seizures with high rates of metabolism, such as convulsive seizures. This fits with the activity of FDP against generalized motor seizures in the post-status epilepticus model. In support of this idea, a decrease in cortical activity in humans [4] and rats [12] during absence seizures has been suggested based on changes in oxygenation of hemoglobin in the cortex during seizures. Thus, during the non-convulsive seizures there is little, or no, increase in metabolic demand within the cortex, so that, if FDP works by shifting metabolism, it would be predicted to be less effective, or ineffective, against non-convulsive seizures. Whether this hypothesis is true remains to be tested.

The time course of the efficacy of FDP in the epilepsy model could have been predicted from the kinetics of FDP in the brain [14]. The half-life of FDP in the brain is about 36 hours, so it would be predicted to reach a steady-state level in about 6 days with oral administration, which is when significant anticonvulsant activity was seen. The delay to the return of seizures after the FDP was removed from the drinking water could also be due to the long half-life of the compound in the brain. After acute administration of 500 mg/kg, FDP reaches maximal levels in the brain (approximately 0.6 mg/g) by 1 hour and these levels are then sustained for about 24 hours [14]. Therefore, FDP would be predicted to be at peak levels at the time of GBL administration. It is possible that efficacy against non-convulsive seizures requires a different dose or dosing regimen than was tested in the current experiments.

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References

1. Abe K, Nakanishi K, Saito H. The possible role of endogenous glutathione as an anticonvulsant in mice. *Brain Res* 2000;854:235–238. [PubMed: 10784128]
2. Aizawa M, Ito Y, Fukuda H. Pharmacological profiles of generalized absence seizures in lethargic, stargazer and γ -hydroxybutyrate-treated model mice. *Neurosci. Res* 1997;29:17–25. [PubMed: 9293489]

3. Bough KJ, Gudi K, Han FT, Rathod AH, Eagles DA. An anticonvulsant profile of the ketogenic diet in the rat. *Epilepsy Res* 2002;50:313–325. [PubMed: 12200222]
4. Buchheim K, Obrig H, Pannwitz W. v. Muller A, Heekeren H, Villringer A, Meierkord H. Decrease in haemoglobin oxygenation during absence seizures in adult humans. *Neurosci Letts* 2004;354:119–122. [PubMed: 14698453]
5. Cavalheiro EA. The pilocarpine model of epilepsy. *Ital J Neurol Sci* 1995;16:33–37. [PubMed: 7642349]
6. Espanol MT, Litt L, Hasegawa K, Chang LH, Macdonald JM, Gregory G, James TL, Chan PH. Fructose-1,6-bisphosphate preserves adenosine triphosphate but not intracellular pH during hypoxia in respiring neonatal rat brain slices. *Anesthesiol* 1998;88:461–472.
7. Kelleher JA, Chan PH, Chan TY, Gregory GA. Energy metabolism in hypoxic astrocytes: protective mechanism of fructose-1,6-bisphosphate. *Neurochem Res* 1995;20:785–792. [PubMed: 7477671]
8. Lian XY, Khan FA, Stringer JL. Fructose 1,6 bisphosphate has anticonvulsant activity in models of acute seizures in adult rats. *J Neurosci* 2007;27:12007–12011. [PubMed: 17978042]
9. Loscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res* 2002;50:105–123. [PubMed: 12151122]
10. Snead OC. Gamma-hydroxybutyrate model of generalized absence seizures: further characterization and comparison with other absence models. *Epilepsia* 1988;29:361–368. [PubMed: 3391142]
11. Snead OC. The γ -hydroxybutyrate model of absence seizures: correlation of regional brain levels of γ -butyrolactone with spike wave discharges. *Neuropharmacol* 1991;30:161–167.
12. Tenney JR, Duong TQ, King JA, Ludwig R, Ferris CF. Corticothalamic modulation during absence seizures in rats: A function MRI assessment. *Epilepsia* 2003;44:1133–1140. [PubMed: 12919383]
13. Vexler ZS, Wong A, Francisco C, Manabat C, Christen S, Tauber M, Ferriero DM, Gregory G. Fructose-1,6-bisphosphate preserves intracellular glutathione and protects cortical neurons against oxidative stress. *Brain Res* 2003;960:90–98. [PubMed: 12505661]
14. Xu K, Stringer JL. Pharmacokinetics of fructose-1,6-diphosphate after intraperitoneal and oral administration to adult rats. *Pharmacol. Res* 2008;234–238. [PubMed: 18325780]

Figure 1.

Effect of oral FDP on spontaneous seizures. The number of spontaneous seizures was measured daily and a baseline frequency was determined by averaging the number of stage 3 (or higher) seizures over a 6 day period. This average seizure frequency was then set at 100% and the number of seizures during each day was normalized to the baseline value in that animal. In the group receiving oral FDP (0.5% in the drinking water), there was a gradual decrease in the number of seizure over the first 7 days of treatment. The drug treatment group was significantly different than the control group (2-way ANOVA, $p < 0.0001$).

Figure 2.

FDP had no effect on spike-wave activity induced with γ -butyrolactone administration. The electrical recording from the cortex of 2 different animals is presented. In A about 10 seconds of baseline activity is presented, followed by 30 seconds of spike-wave activity recorded 5 minutes after administration of 100 mg/kg GBL. In B, a baseline recording was obtained and then the animal received 500 mg/kg FDP. There was no effect of the FDP on the electrical activity in the cortex as shown 30 minutes after administration of FDP. Subsequent administration of GBL resulted in typical spike-wave activity as shown 5 minutes after administration of GBL. Calibrations for all recordings are indicated in the figure.