Fructose-1,6-diphosphate inhibits seizure acquisition in fast hippocampal kindling

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A B S T R A C T

Inhibition of glycolytic metabolism may provide a new therapy for refractory epilepsy. Fructose-1,6-diphosphate (FDP), which inhibits glycolysis and diverts glucose into the pentose phosphate pathway, has strong inhibitory action on seizures induced by chemical convulsants. Here, we investigated the effect of FDP on a rat model of rapid hippocampal kindling. After determining the after-discharge threshold (ADT), the seizure severity and after-discharge duration (ADD) were measured to study the antiepileptogenic effects of FDP (0.5 or 1.0 g/kg i.p. for 4 days). The mRNA expression levels of the brain-derived neurotrophic factor (BDNF) and its principal receptor TrkB, which are key modulators of seizure activity, were determined in the ipsilateral hippocampus by real-time polymerase chain reaction (RT-PCR). High-dose FDP (1.0 g/kg) delayed kindling development together with shortened ADD, and high-dose treated rats also needed more kindling stimulations and more cumulative ADD to stage 4. However, it showed no significant antiepileptogenic effect at a lower dose of 0.5 g/kg. In addition, FDP attenuated BDNF and TrkB expression before and during kindling procedure; this result indicated that BDNF/TrkB signaling pathway may participate in the antiepileptogenic action of FDP. Our data demonstrates that FDP has a significant antiepileptogenic effect in kindling seizures and that it may be a potential antiepileptic drug in the future.

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signaling pathway participates in the antiepilepticogenic action of FDP.

Male Sprague–Dawley rats (weighting 260–300 g) were housed individually and kept on a 12-h light/dark cycle. Under chlo-
ral hydrate anesthesia (400 mg/kg), rats were mounted on a stereotactic apparatus (512600; Stoelting, USA). Electrodes were implanted into the right ventral hippocampus (coordinates from bregma: AP = -5.4 mm, L = -5.2 mm, and V = -6.5 mm). The elec-
rodes were made of twisted. Teflon-coated stainless steel wires (diameter, 0.13 mm; A.M. Systems, USA) insulated except at the tip were used for electrical stimulation and electroencephalographic (EEG) recording. The electrodes were connected to a miniature receptacle, which was attached to the skull with dental cement. After surgery, the rats were allowed to recover for 10 days. All the experiments were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Ani-
mals.

On the first day of stimulation, the after-discharge threshold (ADT) was determined using a 2-s stimulus of 60-Hz monophasic square waves at 1 ms per pulse. The initial stimulus intensity was at 50 μA and was subsequently increased in steps of 20 μA until at least 5 s of after-discharge was elicited. The minimal intensity that produced after-discharge was designated as the ADT for that animal. The stimulation parameters (YC–2; Chengyi, China) were 1-ms square-wave pulses with an intensity of 450 μA at 60 Hz for 10 s. According to previous study [14], the animals were subjected to 12 kindling stimulations per day at 30-min intervals for four consecutive days.

To investigate the antiepileptic effects, 0.5 or 1.0 g/kg i.p. FDP, or saline i.p. were administered (each group characterized by similar mean average ADT values). Based on the pharmacokinetics in the previous literature [22], kindling stimulation to the ventral hip-
locampus was performed 1 h after each i.p. injection. Seizures were graded according to an adjusted version of the Racine scale [12,16]: stage 1, whisker twitching; stage 2, chewing, head bobbing; stage 3, forelimb clonus; stage 4, forelimb clonus and rearing; and stage 5, forelimb clonus, rearing and falling. Stage 4 or 5 was considered kind-
dled motor seizure. EEG at the ventral hippocampus was recorded using a digital amplifier (RM–62160C; Chengyi, China).

The rats were killed by decapitation, the brains were removed 1 h after the last stimulation, and then the ipsilat-
eral hippocampus were dissected and immediately frozen in liquid nitrogen. Total RNA was extracted from the right hippocampal tissues by using the TRIzol reagent (Invitrogen, USA). RT-PCR was carried out using the SYBR Green Real-
time PCR Master Mix (QPK-201; Toyobo, Japan) and the Light Cycler® 2.0 Instrument (Roche Diagnostics Corporation, Germany) according to the manufacturer’s instructions. Primers were designed and synthesized by Invitrogen Biotechnology Co. Ltd. (Shanghai, China). The primers sequences were as following: GAPDH forward: 5′–GGTGACCACTGAGCAGATCAT-3′ and reverse: 5′–GGCTCCTCTTGGCTCAGTATCTT–3′; BDNF forward: 5′–CAGGGCATAGACAAAAG–3′ and reverse: 5′–CTTCTTCTTATTTAGTC–3′; TrkB forward: 5′–TGAGCGAGTCCAGATGC–3′ and reverse: 5′–
TTCTGCTTACATGATCCTT–3′.

At the end of the behavioral experiments, the electrode place-
ments were histologically verified by staining with toluidine blue O. Data from the animals in which the electrodes were placed within the right ventral hippocampus according to the atlas of Watson and Paxinos [15] were included in the statistical analysis. For ani-
mals used in biochemical experiment, the hippocampal electrode placement was examined and confirmed in the process of tissue separation.

Data are presented as mean ± standard error of the mean (S.E.M.). Statistical evaluation of the group differences in kin-
dling acquisition was performed with two-way analysis of variance (ANOVA). Other tests were performed with an analysis of one-way ANOVA when the data were normally distributed and the variances were homogeneous; otherwise, the nonparametric Mann–Whitney U-tests were used as indicated in the figure legends. For all analyses, the tests were two-sided, and a P < 0.05 was considered statistically significant.

Of the 115 rats included in our study, 31 were divided into three groups to investigate the semiology and EEG, and 74 were killed by decapitation to collect samples for molecular analysis. The ani-
mals displayed no signs of abdominal cramps or other abnormal behavior after i.p. injection of FDP.

The electrodes were found in the right ventral hippocampus after behavioral experiments in 31 rats, including the rats pre-
treated with low-dose FDP (0.5 g/kg, n = 9), the rats pretreated with high-dose FDP (1.0 g/kg, n = 12) and the rats pretreated with saline (n = 10). No stage 4–5 seizure was found on the first day in any group. On the second day, stage 4–5 seizure occurred in 10 rats injected with saline and 6 rats pretreated with low-dose FDP but not in those pretreated with high-dose FDP. The mean seizure stage during the whole kindling session was significantly delayed in the rats pretreated with high-dose FDP (P < 0.01) but not significantly modified by low-dose FDP, in comparison to control (Fig. 1).

To analyze the stepwise progression of kindling, the mean seizure stage and cumulative after-discharge duration (ADD) were measured on each day. The high-dose FDP significantly reduced the mean seizure stage on days 2, 3, and 4 (P < 0.01, Fig. 2A); and it shorted the cumulative ADD on day 2 (P < 0.05, Fig. 2B) and day 4 (P < 0.01). Low-dose FDP only decreased the mean seizure stage on day 3 (P < 0.05, Fig. 2A), and did not significantly change the cumulative ADD on any day.

The rats pretreated with 1.0 g/kg of FDP needed approximately 30% more kindling stimulations and 43% more cumulative ADD to reach stage 4 than the rats pretreated with saline (P < 0.01, Fig. 2C, D). Additionally, pretreatment of high-dose FDP reduced the number of stage 5 seizures by 66% on day 4 (P < 0.01, Fig. 2E). In contrast, low-dose FDP (0.5 g/kg) had no such effect.

Of the 74 rats administered with FDP (1.0 g/kg) or saline respec-
tively once per day, we measured the gene expression before (0 Sti, n = 10) and after 5 (5 Sti, n = 10), 24 (24 Sti, n = 8), and 48 (48 Sti, n = 9) stimulations in the hippocampus. The relative abundance of BDNF

Fig. 1. Effects of saline, low-dose FDP (0.5 g/kg), and high-dose FDP (1.0 g/kg) on the behavioral stage of seizures during right ventral hippocampus kindling acquisition. *P < 0.01 compared with the control. n = 10 for control group, n = 9 for 0.5 g/kg FDP group, and n = 12 for 1.0 g/kg FDP group. Two-way ANOVA was used for statistical analysis.
mRNA significantly increased after 5 stimulations \( (P < 0.01, \text{Fig. 3A}) \). This change continued until the completion of 48 stimulations. Consistent with the increase in BDNF levels, there was a significant upregulation of TrkB mRNA after 24 stimulations \( (P < 0.01, \text{Fig. 3B}) \). This upregulation was transient, and the expression returned to the baseline level at the end of 48 stimulations.

FDP had significant inhibitory effect on BDNF expression in absence of any seizure \( (P < 0.01, \text{Fig. 3A}) \); however, the TrkB mRNA level did not significantly change before kindling (Fig. 3B). In animals pretreated with FDP (1.0 g/kg), the BDNF mRNA level was significantly downregulated after 5 kindling stimulations than the control \( (P < 0.01, \text{Fig. 3A}) \), and TrkB mRNA level was conspicuously downregulated after 24 stimulations \( (P < 0.05, \text{Fig. 3B}) \).

Metabolic regulation of neuronal excitability might represent targets for pharmacological intervention to treat drug-resistant epilepsy [7]. Many researches have shown that ketogenic diet is a valuable therapeutic option for patients with intractable epilepsy. However, it has poor tolerability; its safety is a subject of debate in the medical community because it causes hypercholesterolemia and kidney stones; and it requires at least 3 months taking effect. These disadvantages greatly limit the application of ketogenic diet, especially in adults. In contrast, FDP can decrease glycolysis as well as preserve cellular glutathione levels, which is an important free radical scavenger in the mammalian nervous system [21]. A single dose of FDP is sufficient for achieving the required concentration in the brain within 1 h, and this level is sustained for about 24 h [22].
In addition, FDP has been safely used as a myocardial preservation drug for many years [17]. In our experiment, FDP modulated gene expression shortly after kindling; this shows that FDP may take effect rapidly.

Data from the present study suggested that high-dose FDP significantly delayed acquisition of seizures induced by rapid hippocampal kindling. In contrast, the effect of FDP at a lower dose of 0.5 g/kg was much weaker. These results suggested that FDP has a dose dependent antiepileptogenic function. We also found FDP did not change the seizure stage on the first day of kindling. However, a single dose of FDP showed an acute inhibitory action on seizures induced by chemical convulsants [11]. This difference may be explained by the fact that electrical stimulus used for fast kindling is strong (the stimulation duration is long and the stimulation interval is short). Furthermore, the rats pretreated with FDP (1.0 g/kg) required much more kindling stimulations to attain kindled motor seizure [12].

BDNF was significantly upregulated shortly after rapid hippocampal kindling in rats. The role of BDNF in epileptogenesis has been established previously [10]. As a key marker in development of epilepsy, BDNF increases neurotransmitter release and strengthens the excitatory synaptic connection, thus promotes build up of epileptic network. Infusion of BDNF in vivo induces seizures [18], enhances the excitatory synaptic connection, thus promotes build up of epilepsy, BDNF increases neurotransmitter release and strengthens hippocampal kindling in rats. The role of BDNF in epileptogenesis has been investigated in a number of studies [12,13,19].

Kindling is a model of epileptogenesis in which repeated electrical stimulation increases the severity of seizures, leading to a progressive increase in kindled motor seizure [12]. FDP (1.0 g/kg) required much more kindling stimulations to attain kindled motor seizure [12]. This difference of 0.5 g/kg was much weaker. These results suggested that FDP may take effect after expression [3]. Therefore, by inhibiting BDNF/TrkB pathway activation, FDP could reduce neural excitability, thereby delaying fast hippocampal kindling.

In conclusion, we demonstrated that FDP delayed kindling acquisition in a rat model of rapid hippocampal kindling seizures. In addition, our preliminary data showed FDP delayed the upregulation of BDNF and TrkB mRNAs in the hippocampus in kindling, which suggests the BDNF/TrkB signaling pathway participates in the antiepileptogenic action of FDP. Further investigation is needed to examine the spatial and temporal profile of the protein expression of BDNF and TrkB and their downstream signal pathway changes. Besides, an antioxidant property of FDP may also contribute to its action [20]. FDP may be a potential antiepileptic agent in the future.

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References