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Glucose utilization in the brain during acute seizure is a useful biomarker for the evaluation of anticonvulsants: effect of methyl ethyl ketone in lithium-pilocarpine status epilepticus rats

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Abstract

Enhancement of glucose utilization in the brain has been well known during acute seizure in various kinds of animal model of epilepsy. This enhancement of glucose utilization might be related to neural damage in these animal models. Recently, we found that methyl ethyl ketone (MEK) had both anticonvulsive and neuroprotective effects in lithium-pilocapine (Li-pilo) status epilepticus (SE) rat. In this article, we measured the uptake of [^{14}C]2-deoxyglucose ([^{14}C]DG) in the Li-pilo SE and Li-pilo SE with MEK rat brain in order to assess whether the glucose utilization was a useful biomarker for the detection of efficacy of anticonvulsive compounds. Significant increase of [^{14}C]DG uptake (45 min after the injection) in the cerebral cortex, hippocampus, amygdala and thalamus during acute seizure induced by Li-pilo were observed. On the other hand, the initial uptake of [^{14}C]DG (1 min after the injection) in the Li-pilo SE rats was not different from the control rats. Therefore, the enhancement of glucose metabolism during acute seizure was due to the facilitation of the rate of phosphorylation process of [^{14}C]DG in the brain. Pretreatment with MEK (8 mmol/kg) completely abolished the enhancement of glucose utilization in the Li-pilo SE rats. The present results indicated that glucose utilization in the brain during acute seizure might be a useful biomarker for the evaluation of efficacy of anticonvulsive compounds.

Keywords: Glucose utilization; [^{14}C]DG; Methyl ethyl ketone; Lithium-pilocarpine; Seizure; Anticonvulsant

1. Introduction

Marked enhancement of glucose utilization in the brain during acute seizures has been demonstrated in various models of epilepsy in adult rodents and primates [1–3]. This regional hypermetabolism has also been shown to be correlated with the development of neural damage in various animal models [2–5]. The rat model of epilepsy induced by the muscarinic agonist, pilocarpine or lithium-pilocarpine (Li-pilo) is frequently used as an animal model of human temporal lobe epilepsy [6,7]. In adult rats, pilocarpine has been demonstrated to induce status epilepticus (SE) followed by a silent seizure-free phase and then spontaneous recurrent seizures. Neural damage has also been shown in the hippocampus, dentate gyrus hius, priform and entorhinal cortex, amygdala, neocortex and thalamus in these rats [8,9]. During SE induced by pilocarpine, dramatic increase of local cerebral glucose utilization, mainly in forebrain regions that show later neural damage in adult rats has been reported [3,4]. A similar correlation between hypermetabolism and subsequent neural damage during SE induced by Li-pilo has also been reported in the adult rat brain [5]. These previous reports suggested that local cerebral glucose utilization during acute seizures might be a useful biomarker for the assessment of anticonvulsant compounds by in vivo autoradiography as well as animal positron emission tomography studies [10].

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The ketogenic diet is a valuable therapeutic approach for patients with intractable epilepsy. This diet is high in fat content and low in carbohydrate and protein content. Levels of β-hydroxybutyrate, acetoacetate and acetone in blood are elevated during the ketogenic diet, and these ketone bodies enter the brain, partly replacing glucose as a fuel in the brain. Among these ketone bodies, acetone easily passes through the blood brain barrier, and then it can be used as an energy source instead of glucose. Although several hypotheses about the mechanism underlying the effects of a ketogenic diet have been suggested, these have been still controversial [11].

Recently, we found that both methyl ethyl ketone (MEK) and diethyl ketone (DEK) exerted potent anticonvulsive effects in the Li-pilo SE rats. The required doses of MEK and DEK to achieve an anti-seizure effect were lower than acetone (acetone: 25 mmol/kg, MEK: 5 mmol/kg, DEK: 3.5 mmol/kg) [12]. Briefly, pretreatment with either MEK (8 mmol/kg) or DEK (2.5 mmol/kg) produced anticonvulsive effects as assessed by behavioral scoring according to the Racine score. In addition, two-time treatment with MEK (8 mmol/kg 15 min prior to the administration of pilocarpine and 5 mmol/kg 3 h later) completely blocked the spontaneous recurrent seizures recorded by electroencephalography (EEG) and also the neural damage measured 4 weeks after the administration of pilocarpine by histochemistry using hematoxylin–eosin staining.

In this study, [^{14}C]2-deoxyglucose ([^{14}C]DG) uptake in the Li-pilo SE and Li-pilo SE with MEK rat brain was measured using a quantitative autoradiographic technique in order to assess whether glucose utilization could be used as a biomarker for the evaluation of anticonvulsants.

2. Materials and methods

2.1. Animals

Male Wistar rats (8–9 weeks old) were purchased from Japan SLC (Shizuoka, Japan). All rats were given free access to food and water. All the animal experiments in this study were carried out with the approval of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University. Total number of rats used in this study was 55.

2.2. Chemicals

[^{14}C]DG (specific radioactivity, 2.04 GBq/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA).

Lithium chloride, pilocarpine hydrochloride and MEK were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity grade available commercially.

2.3. Treatment with chemicals

Control: rats were received lithium chloride (3 mEq/kg ip) and an equal volume of saline instead of MEK. MEK-treated; Rats were received lithium chloride and MEK (8 mmol/kg ip; diluted with saline solution: 10 ml/kg). Li-pilo SE: rats were received lithium chloride (3 mEq/kg ip) 20–24 h prior to injection of the pilocarpine hydrochloride (32 mg/kg sc), and then, an equal volume of saline instead of MEK was injected 15 min prior to the administration of pilocarpine hydrochloride. Li-pilo SE with MEK: rats received lithium chloride (3 mEq/kg ip), and then, MEK (8 mmol/kg ip; diluted with saline solution: 10 ml/kg) was injected 15 min prior to the administration of pilocarpine hydrochloride (32 mg/kg sc).

2.4. Behavioral scoring of the Li-pilo SE and Li-pilo SE with MEK rat

After the pilocarpine injection, the rats were isolated in plastic cages and their behavior was observed. The presence or absence of any type of seizure activity was scored according to a previously described method [13]: Stage 1 seizures consisted of immobility and occasional facial clonus, Stage 2 seizures included head nodding, Stage 3 seizures were associated with bilateral forelimb clonus, Stage 4 seizures included rearing and Stage 5 seizures were associated with rearing and falling.

2.5. Autoradiography using [^{14}C]DG in the control, MEK-treated, Li-pilo SE and Li-pilo SE with MEK rat brains

Rats in Li-pilo SE group were intravenously injected with [^{14}C]DG (185 kBq) 90 min after the beginning of the seizure (Stage 4) and then decapitated at 1 min or 45 min post injection of the tracer. Other rats (control, MEK-treated and Li-pilo SE with MEK) were intravenously injected with [^{14}C]DG (185 kBq) 120 min after the administration of saline, MEK or pilocarpine solution. The brains were quickly removed and frozen sections (20 μm) were prepared. The sections were exposed to imaging plates (Fuji Film, Tokyo, Japan) for 1–4 weeks, and autoradiograms were generated using a bioimaging analyzer (BAS1800; Fuji Photo Film, Tokyo, Japan). Regions of interest (ROIs) were drawn on the images, and the radioactivity concentration in each ROI was expressed as photostimulated luminescence per square millimeter area (PSL/mm^2). Then, the PSL/mm^2 values were converted to a percentage of the total injected dose of the tracer per gram of tissue (% dose/g) using [^{14}C] microscales (Amersham Biosciences, UK).

2.6. Statistical analysis

Statistical analysis was performed by Student's *t* test.