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Fasting time duration modulates the onset of insulin-induced hypoglycemic seizures in mice

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ABSTRACT

Objective: Fasting (48 h) in mice causes resistance to insulin-induced hypoglycemic seizures (IIHS) but in rats fasting (14–16 h) predisposes IIHS. So we suspect the duration of fasting may possibly affect the onset of seizures and in this study, we investigated the IIHS by administering 8 Units (U) insulin (INS)/k.g., intraperitoneally to 8 weeks old male C57BL6/J mice.
Methods: The mice were divided into group 1 (non-fasted), group 2 (6 h fasted) and group 3 (24 h fasted) and we administered the 8 U INS. The first behavioral hypoglycemic seizure symptoms such as jump, clonus or barrel rotations considered as seizure onset and we analyzed the blood glucose level (BGL) and serum beta-hydroxybutyrate (BHB) level.
Results: The time of first seizure onset in group 1 was 109.7 ± 4.3 min, group 2 was 46.50 ± 3.9 min and group 3 was 165.4 ± 13.26 min. The seizure onset time in group 2 was significantly decreased compared to group 1. The seizure onset time in group 3 was significantly increased compared to group 1 and group 2. The decreased BGL after INS administration was correlated with the seizure onset time in group 1 and group 2 but not in group 3. The BHB level in group 3 was significantly higher compared to group 1 and 2.
Conclusion: Our data show that the fasting time duration significantly modulates the onset of hypoglycemic seizures. The opposite effect of 6 h or 24 h fasting time duration is likely caused by different BHB levels.

1. Introduction

Acute symptomatic seizures are seizures that having a temporal association with documented systemic brain insults that may be metabolic, structural, toxic, infectious or inflammatory in nature. The metabolic insults caused by glucose or electrolyte disturbances, endocrine dysfunction produce seizures in critically ill patients (Imad et al., 2015; Vaughan and Delanty, 2002).

Hypoglycemia is one of the frequent complaints in the emergency department (Ford et al., 2013). In a 6.5 years follow in Diabetic Control and Complication Trail, there were 1,027 episodes of coma or seizures (The Diabetes Control and Complications Trial Research Group, 1997).

During severe insulin-induced hypoglycemia, the brain energy metabolite or reserve such as pyruvate or adenosine triphosphate (ATP) were decreased (Cardell et al., 1991). Chronic ketogenic diet upregulates the energy metabolism enzymes and it increases ATP

Abbreviations: ATP, adenosine triphosphate; BGL, blood glucose level; F, fasting; *i.p.*, intraperitoneally; H, hour; IIHS, insulin induced hypoglycemic seizures; INS, insulin; NMDA, *N* methyl D aspartate; U, unit; USP, United States Pharmacopeia.

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levels during seizures (Bough et al., 2006; Nakazawa et al., 1983). Chronic ketosis decreases the cerebral metabolic rate of glucose and the increase in ketone body utilization in the brain is considered as to have an anticonvulsive property (Giménez-Cassina et al., 2012; Zhang et al., 2013a).

Fasting decreases the blood glucose level (BGL) and it increases the alternate fuel ketone bodies such as beta-hydroxybutyrate (Kerndt et al., 1982). The increased level of beta-hydroxybutyrate in fasting may play an important role in seizure protection (Yum et al., 2012). During insulin-induced hypoglycemic seizures (IIHS), 48 h fasted mice were resistant to IIHS. Contrary to the seizure-protective effect by 48 h fasting, 14–16 h fasted rats predisposes IIHS (Tamasi and Drenick, 1973; Velísek et al., 2008).

This raises the question that, why fasting increases (anticonvulsant) and decreases (proconvulsant) seizure onset time after insulin-induced severe hypoglycemia in mice and rat respectively? We suspect that the time duration of fasting may play a role in the seizure onset. So in this study, we investigated the effect of 8 U IIHS in the non-fasted group and two fasted group (6 h and 24 h) in mice. Our results suggest that the fasting BHB level might determine the seizure onset time during severe hypoglycemia.

2. Materials and methods

2.1. Animals

We used 8 weeks old male C57BL6/J mice. The mice were housed in 12:12 h light: dark cycle schedule with a controlled temperature 23 ± 2 °C and humidity 55 ± 15%. We divided them into three groups. Group 1 mice (non-fasted) had free access to food and water (n = 9), group 2 mice (6 h fasted) had free access to water but not food for 6 h (n = 10) and group 3 mice (24 h fasted) had free access to water but not food for 24 h (n = 8). All experiments with animals were performed in accordance with the national guidelines and approved by the animal care committee of Niigata University of Pharmacy and Applied Life Sciences (Approval H2703-3), Niigata, Japan.

2.2. Insulin dose and administration

We used pancreatic insulin (Sigma I0516 10 mg/ml) derived from bovine source (Clodfelder-Miller et al., 2005). It contains not less than 27 USP U/mg and based on the manufacturer's instructions that some activity may lose during the manufacturing process, we calculated the dose of 24 U/mg. So we have chosen 0.33 mg/kg insulin, which is equivalent to 8 U/kg insulin dissolved in normal saline and administered intraperitoneally (*i.p*). The administration of insulin injection was considered as 0 min and we observed the behavioral seizures symptoms for up to 4 h duration in all the groups.

2.3. Seizure scoring and seizure onset

The seizures scores were calculated based on previously reported hypoglycemic seizure scores (Maheandiran et al., 2013) and modified by our behavioral observations (Table 1). The first symptoms of the jump, clonus or barrel rotation was considered as seizure onset. The seizure symptoms or behaviors were not videotaped and it was not a blinded observation.

2.4. Blood collection

Blood samples were collected from tail bleeds and BGL was checked using Freestyle Freedom glucometer. For the beta-hydroxybutyrate assay, the mice were anesthetized with pentobarbital 50 mg/kg (*i.p*) and the blood was collected using retro-orbital puncture. The serum was separated by centrifugation (3000*g*) for 15 min at 4° C and stored at −80° C.

Table 1
This table represents the seizures score based on the behavior symptoms.

Score	Behavior symptoms
0.5	Tail Flicking or Tail rotation
1	All legs extended and the body touches the ground
1.5	Shivering
2	Hind leg pushing down and the body extending upward
2.5	Running
3	Head bend backwards
3.5	Tonic extension
4	Jumping
4.5	Unilateral forelimb or hind limb clonus
5	Barrel Rotation
5.5	Severe continuous clonus till death

Table 2
This table represents the body weight. In group 2 (non-fasted vs 6 h fasted) and group 3 (non-fasted vs 24 h fasted) the body weight was significantly decreased (*p < 0.05 and ***p < 0.001). Results are mean ± S.E.M. Data were analyzed using Unpaired *t*-test.

	Body weight (g)		
	Non fasted	6 h fasted	24 h fasted
Group 1	23.77 ± 0.8		
Group 2	22.89 ± 0.3	21.79 ± 0.3 *	
Group 3	22.69 ± 0.4		18.86 ± 0.56 ***

2.5. Beta-hydroxybutyrate assay

We used separate mice for non-fasted, (n = 4), 6 h fasted, (n = 5) and 24 h fasted, (n = 5) for the beta-hydroxybutyrate assay. The serum samples were analyzed using Bio-vision beta-hydroxybutyrate colorimetric assay kit (K632-100).

2.6. Statistical analysis

Statistical analysis was performed using a two-tailed Unpaired *t*-test, one-way ANOVA followed by Tukey's test and Fishers exact test.

3. Results

3.1. Body weight

The initial body weight among group 1 (23.77 ± 0.8 g), group 2 (22.89 ± 0.3 g) and group 3 (22.69 ± 0.4 g) were not significantly different. The group 2 mice fasted for 6 h and the body weight was significantly decreased ($p < 0.05$) from 22.89 ± 0.3 to 21.79 ± 0.3 g. The group 3 mice fasted for 24 h and the body weight was significantly decreased ($p < 0.001$) from 22.69 ± 0.4 to 18.86 ± 0.56 g (Table 2).

3.2. Blood glucose level

The initial BGL among group 1 (161.4 ± 6.58 mg/dl), group 2 (163.6 ± 4.2 mg/dl) and group 3 (164.8 ± 4.1 mg/dl) mice were not significantly different. The group 2 mice fasted for 6 h and the BGL was significantly decreased ($p < 0.05$) from 163.6 ± 4.2 to 121.1 ± 5.9 mg/dl. The group 3 mice fasted for 24 h and the BGL was significantly decreased ($p < 0.001$) from 164.8 ± 4.1 to 74.63 ± 4.5 mg/dl (Table 3).