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Research Paper

Hypothalamic AMP-Activated Protein Kinase Regulates Biphasic Insulin Secretion from Pancreatic β Cells during Fasting and in Type 2 Diabetes

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ABSTRACT

Glucose-stimulated insulin secretion (GSIS) by pancreatic β cells is biphasic. However, the physiological significance of biphasic GSIS and its relationship to diabetes are not yet fully understood. This study demonstrated that impaired first-phase GSIS follows fasting, leading to increased blood glucose levels and brain glucose distribution in humans. Animal experiments to determine a possible network between the brain and β cells revealed that fasting-dependent hyperactivation of AMP-activated protein kinase in the hypothalamus inhibited first-phase GSIS by stimulating the α-adrenergic pancreatic nerve. Furthermore, abnormal excitability of this brain-β cell neural axis was involved in diabetes-related impairment of first-phase GSIS in diabetic animals. Finally, pancreatic denervation improved first-phase GSIS and glucose tolerance and ameliorated severe diabetes by preventing β cell loss in diabetic animals. These results indicate that impaired first-phase GSIS is critical for brain distribution of dietary glucose after fasting. Furthermore, β cells in individuals with diabetes mistakenly sense that they are under conditions that mimic prolonged fasting. The present study provides additional insight into both β cell physiology and the pathogenesis of β cell dysfunction in type 2 diabetes.

1. Introduction

Insulin has many physiological roles, including anabolic action on dietary energy storage, which is important for overcoming upcoming fasting periods (Cahill, 1971). However, the anti-catabolic action of insulin must be suppressed during fasting to avoid a life-threatening hypoglycemic event. Therefore, the precise systems that monitor fasting or feeding states and subsequently control insulin action are essential for maintaining whole-body glucose homeostasis to overcome fasting. Dysregulation of these systems contributes to type 2 diabetes pathogenesis (Kadowaki, 2000; Kahn and White, 1988; Kulkarni et al., 1999; Seino et al., 2011). Therefore, more complete knowledge of the relationship between insulin physiology and fasting will contribute to a better understanding of type 2 diabetes and may lead to effective therapeutic options.

Abbreviations: ACC, acetyl CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside; AIR, acute insulin response; AMPK, AMP-activated protein kinase; CT, computed tomography; 2-DG, 2-deoxy-D-glucose; ^{18}F-FDG, 2-[fluorine-18]-2-deoxy-D-glucose; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HOMA-β, homeostasis model assessment beta-cell function index; ICV, intracerebroventricular; IPITT, intraperitoneal insulin tolerance test; FSIVGTT, frequently sampled intravenous glucose tolerance test; Kg, glucose disappearance rate; LETO, Long-Evans Tokushima Otsuka; OGTT, oral glucose tolerance test; OLETF, Otsuka Long-Evans Tokushima Fatty; PET, positron emission tomography; PNx, pancreatic denervation; SD, Sprague-Dawley; SNPs, single nucleotide polymorphisms; SNS, sympathetic nervous system; SUV, standardized uptake value.

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Glucose-stimulated insulin secretion (GSIS) from pancreatic β cells is biphasic (Porte and Pupo, 1969; Curry et al., 1968; Seino et al., 2011). Since the first report of biphasic GSIS by Curry et al. in 1968, the dynamics of biphasic GSIS have been examined under both normal physiological conditions and in the pathogenesis of type 2 diabetes (Pfeifer et al., 1981; Polyzogopoulou et al., 2003; Weyer et al., 1999). The current consensus holds that a deficient first-phase GSIS as determined by a frequently sampled intravenous glucose tolerance test (FSIVGTT) represents the earliest identifiable change to β cells in the course of type 2 diabetes. First-phase GSIS in the FSIVGTT is closely associated with the early insulin response to oral glucose ingestion (Caumo and Luzi, 2004; Basu et al., 1996). Furthermore, the loss of an early insulin response is involved in glucose intolerance in diabetes (Basu et al., 1996; Calles-Escandon and Robbins, 1987; Mitrakou et al., 1992). Therefore, the quality of biphasic GSIS control may represent a therapeutic target for better glycemic regulation in type 2 diabetes (Seino et al., 2011; Caumo and Luzi, 2004). However, the mechanisms underlying diabetes-related abnormal biphasic GSIS are not yet fully understood.

Interestingly, fasting also inhibits first-phase GSIS (Fink et al., 1974; Grey et al., 1970). This suggests that fasting-dependent changes in first-phase GSIS may affect glucose metabolism even in non-diabetic individuals. Given that glucose distribution is independent of insulin action in the brain but insulin-dependent in peripheral tissues, such as skeletal muscle and adipose tissue, changes in first-phase GSIS may influence dietary glucose distribution to tissues during re-feeding. Decreased first-phase GSIS after fasting may accelerate a passive glucose distribution to the brain, whereas enhanced first-phase GSIS during frequent feeding could stimulate glucose uptake and anabolism in insulin-sensitive tissues. This may represent an important physiological role of first-phase GSIS in whole-body glucose homeostasis during re-feeding.

In this study, we hypothesized that biphasic GSIS is an adaptive system that appropriately distributes dietary glucose to either the brain or insulin-sensitive tissues, depending on the last fasting period. Furthermore, we proposed that both diabetes- and fasting-related inhibition of first-phase GSIS are regulated by a common system. We demonstrated that hypothalamic AMP-activated protein kinase (AMPK) senses fasting periods and controls first-phase GSIS via the sympathetic nervous system (SNS). Additionally, dysregulation of this system is involved in β cell dysfunction in type 2 diabetes.

2. Materials and Methods

2.1. Study Approvals

All human study volunteers provided written informed consent. The study protocol was approved by the Scientific-Ethical Committees of Shiga University of Medical Science and adhered to the guidelines of the Declaration of Helsinki regarding ethical principles for medical research involving human subjects. Additionally, all procedures with animals were performed in accordance with the guidelines of the Research Center for Animal Life Science of Shiga University of Medical Science and followed the guidelines provided by the Animal Research: Reporting In Vivo Experiments (ARRIVE) guideline.

2.2. Human ^{18}F-FDG-PET-CT Analysis

Fig. S1A displays a schematic of the protocol for 2-[fluorine-18]-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET)-computed tomography (CT) and intravenous glucose infusion after 3 and 12 h of fasting in three healthy volunteers. We chose 3-h and 12-h fasts to represent physiologically relevant timeframes from daily life. These intervals correspond to common intervals between breakfast and lunch and between dinner and breakfast, respectively. We performed ^{18}F-FDG-PET-CT on the three volunteers after the 3-h and 12-h fasts. The interval between the two examinations was 2 weeks. For the 12-h fasting condition, all volunteers were instructed to refrain from smoking and drinking coffee or alcoholic beverages from 9:00 pm on the night before the study. For the 3-h fasting condition, the volunteers received oral glucose (75 g) at 6:00 am and were placed in a comfortable supine position. The experiments began at 9:00 am. Glucose (0.3 g/kg) was administered intravenously over 1.5 min, followed by intravenous injection of ^{18}F-FDG. Sequential scans of the brain were obtained at 3-min intervals during the first 30 min after the injections. Whole-body scans were obtained 120 min after glucose and ^{18}F-FDG injections. The standardized uptake value was analyzed to quantify the accumulated ^{18}F-FDG in the indicated tissues (Lucignani et al., 2004).

2.3. Human FSIVGTTs

Fig. S1B displays a schematic of the FSIVGTT performed in 40 healthy volunteers after fasting for 3 or 12 h. The fasting and meal conditions were the same as those described in Section 2.2. At 9:00 am, fasting blood samples were collected. We then administered 0.3 g/kg glucose intravenously over 1.5 min, and blood was collected after 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 27, 32, 42, 52, 62, 72, 82, 92, 102, 112, and 122 min. The acute insulin response (AIR) and glucose disappearance rate (Kg) were evaluated with MINMOD Millennium (Pacini and Bergman, 1986; Boston et al., 2003). Blood glucose, plasma insulin, free fatty acid, and hemoglobin A1c (NGSP value) concentrations were measured using standard laboratory methods. The active forms of glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide were measured using cell-based bioassay methods (Yanagimachi et al., 2016). The mean values of these parameters as well as clinical characteristics, including gender, age, body weight, body mass index, homeostasis model assessment of insulin resistance, homeostasis model assessment of beta cell function (HOMA-β), and blood pressure, in the 40 volunteers are displayed in Table S1.

2.4. Hyperglycemic (HG) Clamping

HG clamping was performed using standard methodology in 12 of the 40 volunteer subjects who had undergone FSIVGTTs. Plasma insulin concentrations at 120 min after glucose infusion initiation were defined as steady-state insulin levels. The clamped glucose level for evaluating insulin secretory capacity was 200 mg/dL. Time-dependent changes in mean glucose and insulin concentrations in the 12 subjects are displayed in Figs. S2A and B.

2.5. Genomic DNA Extraction and Single Nucleotide Polymorphism (SNP) Genotyping

Genomic DNA samples were harvested from peripheral blood leukocytes from 40 healthy subjects following a standard protocol. Individual SNP genotypes were analyzed using a multiplex-PCR invader assay (Ohnishi et al., 2001). We selected SNPs associated with insulin secretory capacity among those associated with diabetes development. These SNPs are listed in Table S2. The accumulated number of risk alleles for impaired insulin secretion was defined as genetic risk score (GRS).

2.6. Animal Care

Male Sprague-Dawley (SD), Long-Evans Tokushima Otsuka (LETO), and Otsuka Long-Evans Tokushima Fatty (OLETF) rats were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). Experimental rats were individually housed in a regulated environment (22 ± 2 °C, 55% ± 10% humidity, 12:12 h light/dark cycle with light on at 7 am). Food and water were available ad libitum except as otherwise indicated. During long-term fasting experiments up to 72 h, rats were able to freely access drinking water. We monitored their physical condition at 6-h intervals. Throughout the study, none of the fasted rats displayed signs of wasting.

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