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Characterization of the molecular mechanisms involved in the increased insulin secretion in rats with acute liver failure

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Abstract

To investigate the mechanism of hyperinsulinaemia in rats with acute liver failure induced by the administration of D-galactosamine (GalN), we focused on the role of polyprimidine tract-binding protein (PTB) in islet insulin synthesis. Recent reports indicate that PTB binds and stabilizes mRNA encoding insulin and insulin secretory granule proteins, including islet cell autoantigen 512 (ICA512), prohormone convertase 1/3 (PC1/3), and PC2. In the present study, glucose-stimulated insulin secretion was significantly increased in GalN-treated rats compared to controls. Levels of mRNA encoding insulin 1, ICA512, and PC1/3 were increased in the pancreatic islets of GalN-treated rats. This mRNA level elevation was not prevented by pretreatment with actinomycin D. When the PTB-binding site in insulin 1 mRNA was incubated with the islet cytosolic fraction, the RNA–protein complex level was increased in the cytosolic fraction obtained from GalN-treated rats compared to the level in control rats. The cytosolic fraction obtained from pancreatic islets obtained from GalN-treated rats had an increased PTB level compared to the levels obtained from the pancreatic islets of control rats. These findings suggest that, in rats with acute liver failure, cytosolic PTB binds and stabilizes mRNA encoding insulin and its secretory granule proteins.

Keywords: Insulin mRNA; Insulin secretory granule protein mRNAs; Polypyrimidine tract-binding protein; Hyperinsulinaemia; Acute liver failure

1. Introduction

It has long been recognized that liver cirrhosis is associated with impaired glucose metabolism. Patients with liver cirrhosis commonly have increased fasting and glucose-induced insulin levels [1–6]. Studies indicate that hyperinsulinaemia in cirrhotic patients is related to increased pancreatic insulin secretion [2–6] and to decreased hepatic insulin extraction [1,2,4]. However, several studies suggest that, in cirrhotic patients, reduced hepatic extraction of insulin only occurs in the advanced stages of the disease [3,6]. Patients with acute liver failure (ALF) also exhibit severe alterations of glucose metabolism, including insulin resistance [7–11] and increased pancreatic insulin secretion [8–11]. Improving the hyperinsulinaemia of patients with ALF may help prolong their survival and shorten their recovery period. Furthermore, studies of patients with ALF suggest that insulin biosynthesis is activated in the early stage of liver disease [8–11]. In addition, in an animal ALF model, islet insulin content and biosynthesis were found to be significantly increased [12]. However, the mechanisms related to this increase in insulin biosynthesis associated with ALF have not yet been established.

Glucose is the main regulator of insulin biosynthesis [13]. Recent findings indicate that glucose promotes the rapid nucleocytoplasmic translocation of polypyrimidine tract-binding protein (PTB) in rat pancreatic islets and insulinoma INS-1 cells [14]. Cytosolic PTB binds and stabilizes both mRNA encoding insulin [15] and insulin secretory granule proteins, including islet cell autoantigen 512 (ICA512), prohormone convertase 1/3 (PC1/3), and PC2 [14], which promotes their translation. These findings suggest that PTB is a key factor that

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posttranscriptionally regulates insulin biosynthesis and insulin secretion. In the present study, we investigated the roles that PTB plays in regulating the expression of the genes encoding insulin and its secretory granule proteins in ALF.

2. Materials and methods

2.1. Animals and experimental design

The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of Tokushima University. Male Sprague–Dawley rats (weight, about 200 g) were housed under a 12-h light/dark cycle, and had free access to a commercially available diet and water. After the rats were deprived of food for 12 h, one group (GalN-treated rats) was injected intraperitoneally with D-galactosamine (GalN, Sigma, St. Louis, MO) dissolved in phosphate-buffered saline (PBS) at a single dose of 400 mg/kg body weight. The control rats were injected with PBS. Some of the GalN-treated rats were given an intraperitoneal injection of actinomycin D (Sigma) at a dose of 750 μg/kg body weight 1 h before the GalN injection. Twelve hours after the GalN (or PBS) injection, the rats were anesthetized by an intraperitoneal injection of pentobarbital, and pancreatic islets were isolated by collagenase digestion of the pancreas according to the method of Gotoh et al. [16]. Briefly, after the distal end of the bile duct was clamped, 12 mL of collagenase solution (typeXI, Sigma, 0.32 mg/mL) were injected into the common bile duct. The distended pancreas was excised and transferred into a 50-mL plastic tube. After incubation for 40 min at 37 °C, cold Hanks' balanced salt solution (HBSS) was added. The plastic tube was gently vortexed and then centrifuged. The pellet was washed gently three times with cold HBSS. Next, the tissue suspension was passed through a mesh filter to remove large undigested tissue. The filtered tissue was then washed again and the pellet was resuspended in 4 mL of a 25% Ficoll solution (GE Healthcare, Piscataway, NJ). The tissue suspension was overlaid with a discontinuous Ficoll gradient (23%, 20.5%, and 11%, 2 mL of each), and this was followed by centrifugation at 800×*g* for 10 min. The tissue at the 20.5/11 interface was collected and washed with HBSS. The isolated islets were immediately used for sample extraction.

2.2. Measurement of plasma alanine aminotransferase (ALT), glucose, and insulin

The plasma ALT concentration was measured by the LDH-UV method using an autoanalyzer. The plasma concentration of glucose and insulin were measured using the Glucose-CII test (Wako, Osaka, Japan) and ELISA Insulin Kit (Morinaga, Yokohama, Japan), respectively.

2.3. Oral glucose tolerance test

Twelve hours after the GalN (or PBS) injection, some of the rats were given a 20% (W/V) glucose solution at a dose of 2 g/kg body weight by oral gavage. At 0, 30, 60, and 120 min after this glucose loading, blood was drawn from the tail vein and used for plasma glucose and insulin assays.

2.4. Quantitative real-time RT-PCR

Total RNA was extracted from pancreatic islets using the ISOGEN system (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol. The first-strand cDNA was synthesized using a first-strand synthesis kit (Invitrogen Corp., Carlsbad, CA). Real-time RT-PCR (LightCycler, Roche Diagnostics, Indianapolis, IN) was done using a QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA). Each sample was analyzed in triplicate. The specific primers used for real-time RT-PCR are shown in Table 1. The mRNA levels were normalized to the level of 18S ribosomal RNA (rRNA).

2.5. Preparation of cell extracts

Cell extracts were prepared using the method described by Andrews et al. [17]. Islets prepared from 3 individual rats in the same group were pooled and suspended in 100 μl of cold buffer A (10 mM HEPES–KOH (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride). The cells were allowed to swell on ice for 10 min, and then vortexed for 10 s. Samples were centrifuged for 10 s, and then the pellet was resuspended in PBS (nuclear fraction). The supernatant fraction was recentrifuged at 13,000×*g* for 10 min at 4 °C. This supernatant fraction was used as the cytosolic fraction. Protein concentration was analyzed using the BCA protein assay reagent (Pierce, Rockford, IL).

2.6. RNA mobility shift analysis

The construction of the insulin 1 cRNA expression vector and the preparation of the cRNA probe were done as previously described [18]. The RNA probe (10,000 cpm) was denatured at 70 °C for 10 min and then gradually cooled to room temperature. The binding reaction was performed with the cytosolic fraction and the RNA probe placed in a binding buffer containing 10 mM HEPES (pH 7.9), 5 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol and 10% glycerol at 30 °C for 30 min. Following digestion with 5 units of RNase T1 (Roche Diagnostics), the samples were separated by 5% PAGE. The dried gel was analyzed using a Fuji BAS-1500 system.

2.7. Western blot analysis

Western blot analysis was carried out as described previously [19]. After blocking, the membranes were incubated with anti-PTB antibody (Zymed Laboratories Inc., CA) or anti-tubulin antibody (Sigma) overnight at 4 °C.

Table 1
Sequences of primers used for real-time RT-PCR

		Sequence	Position
Insulin 1 (**J00747**)	Sense	ACAGCACCTTTGTGGTCC	4262–4579
	Antisense	GGACTCAGTTGCAGTAGTTC	4518–4499
ICA512 (**NM_053881**)	Sense	TGCGCTCATTGCTGCTTACTCTG	1733–1755
	Antisense	GGCGCTCCTTATCCCGTTGTTT	1846–1825
PC1/3 (**NM_017091**)	Sense	GTACCCAAAAACTCCAGCAG	2143–2162
	Antisense	GGCTTGTTGAGCTTTTCCAG	2345–2326
CPE (**M31602**)	Sense	TTGGGAACATGCATGGTAATGAG	388–410
	Antisense	CACGAACCAGTCCTTCAGCTC	590–570
β-actin (**BC063166**)	Sense	GCCCTGGCTCCTAGCACC	1036–1053
	Antisense	CCACCAATCCACACAGAGTACTTG	1109–1086
18S rRNA (**M11188**)	Sense	AGTCCCTGCCCTTTGTACACA	1691–1711
	Antisense	GATCCGAGGGCCTCACTAAAC	1758–1738

The GenBank accession numbers are indicated in brackets. The primer sequences are written from 5′ to 3′ end.