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Research paper

Changes of the expressions of multiple metabolism genes in rat pancreatic islets after ventromedial hypothalamic lesioning

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HIGHLIGHTS

- VMH lesions change the expression of metabolism genes in rat pancreatic islets.
- VMH lesions upregulated genes involved in hormone activity.
- VMH lesions downregulated genes involved in response to hormone stimulus.

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ABSTRACT

It was recently reported that ventromedial hypothalamic lesions change the expression of cell proliferation-related genes and morphology-related genes in rat pancreatic islets. This study has examined how gene families involved in metabolism are regulated in rat pancreatic islets after VMH lesions formation. Total pancreatic islets RNA was extracted, and differences in the gene expression profiles between rats at day 3 after VMH lesioning and sham–VMH-lesioned rats were investigated using DNA microarray and real-time polymerase chain reaction. The VMH lesions regulated the genes that are involved in functions related to metabolism in the pancreas islets. Real-time polymerase chain reaction also confirmed that gene expressions of arachidonate 15-lipoxygenase (*Alox15*) was up-regulated and pancreatic lipase (*Pnlip*) was downregulated at day 3 after the VMH lesions. Ventromedial hypothalamic lesions may change the expression of multiple metabolism genes in rat pancreatic islets.

1. Introduction

The islets of pancreas form a nutrient sensing network spread throughout the pancreas [20]. The expression of a unique set of proteins enables β cells, the most frequent islet cell type, to detect elevated blood glucose levels and secrete insulin accordingly. Neighboring β-cells achieve tighter regulation of glucose-induced insulin secretion by coordination through cell surface proteins. They also adjust their secretory pathway capacity and flow to avoid being damaged. The immediate reaction of the β cell to nutrients is regulated by translational mechanisms, while longer term adaptations involve changes in transcription. Glucose increases protein synthesis in the β cell overall and especially that of some secretory proteins including insulin. While, intracellular glucose signaling pathways control the secretion of glucagon and insulin by pancreatic islet α- and β-cells, respectively. However, glucose also indirectly controls the secretion of these hormones through regulation of the autonomic nervous system that richly innervates this endocrine organ. Both parasympathetic and sympathetic nervous systems also impact endocrine pancreas postnatal development and plasticity in adult animals [21].

We previously reported that ventromedial hypothalamic (VMH) lesions stimulated cell proliferation of rat pancreatic islet B and acinar cells primarily through a cholinergic receptor mechanism [8], and changed the expression of cell proliferation-related genes and morphology-related genes in rat pancreatic islets [6]. DNA microarray analysis is a powerful tool for detecting the characterization of the messenger RNA (mRNA) expression pattern of a large number of genes. In the present study, we used DNA microarray analysis to identify multiple metabolism genes for which expression profiles showed significant modulation and to investigate the cellular

Abbreviations: Alox15, arachidonate 15-lipoxygenase; cDNA, complementary DNA; DAVID, Database for Annotation, Visualization, and Integrated Discovery; PCR, polymerase chain reaction; Pnlip, pancreatic lipase; Stc1, stanniocalcin; VMH, ventromedial hypothalamus.

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mechanisms of gene regulation in the rat pancreatic islets alone at day 3 after VMH lesions, because it has been reported that cell proliferation in the pancreas increases and reaches a maximum at day 3 [8]. Real-time polymerase chain reaction (PCR) also confirmed a part of the results obtained by DNA microarray analysis.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats weighing 230–250 g were used in this study. They were maintained in a constant temperature environment (23 ± 2 °C) in light-controlled cages with a 12-h light–dark cycle (lights on at 7:00 AM) and were given free access to food and water. Tissue samples were taken from the pancreata of VMH-lesioned rats and sham–VMH lesioned rats at day 3 after the operation ($n = 2$ in each group for DNA chips and $n = 3$ in each group for real-time PCR).

All animal procedures were performed in accordance with the Guidelines for Animal Experiments at the National Hospital Organization (NHO) Kure Medical Center and Chugoku Cancer Center. The protocols were approved by the Animal Research Ethics Committee, NHO Kure Medical Center and Chugoku Cancer Center.

2.2. VMH lesions

Ventromedial hypothalamic lesions or simulated operations were performed as previously described [8]. After the operations, the rats were returned to their cages and given free access to food and water. Localization of the VMH lesions was verified by microscopic examination of the brain at the end of the experiment.

2.3. RNA extraction from rat pancreatic islets

In two individual rats at day 3 after VMH lesioning and two sham–VMH-lesioned rats, to circumvent RNA lysis by RNases that may be released in the rat pancreas when the animal is stressed, all procedures were conducted as swiftly as possible after each rat was killed. The abdominal and chest cavities were opened. The isolation of intact RNA from the rat pancreas was compromised by autolysis and the presence of endogenous ribonucleases [17]. We recently described a technique that reliably improves the amount and the quality of RNA extracted from rat pancreatic islets [9]. Dithizone is a selective stain for pancreatic islets that facilitate their identification [18]. Islet equivalence was determined by staining with 1 mg/mL dithizone, waiting 5 min and observing islets at 25× on a standard stereomicroscope. Immediately after purification, islets were used for RNA extraction. The RNA was quantified spectrophotometrically at 260/280 nm, and the quality of the isolated total RNA was determined by electrophoretic separation on anthodium bromide containing 1 % agarose gel.

2.4. DNA microarray analysis

The preparation of complementary RNA was carried out by Ambion's WT expression (Life Technologies Japan Co., Tokyo, Japan), and target hybridization was performed according to instructions provided in the Affymetrix GeneChip technical manual, as described previously [7]. In the present study, the double-stranded complementary DNA (cDNA) was comprised of hybridized Affymetrix GeneChip arrays (Rat Gene 1.0 ST Array; Affymetrix Japan Co., Tokyo, Japan). The digitized image data were processed using GeneChip Operating Software 1.4 (Affymetrix Japan Co.). The amount of probe-specific transcripts was determined as per the average of differences between the perfectmatch and mismatch intensities. We identified genes with fold-changes >2.0 as differentially expressed genes. As replicate assays were not performed, a very stringent cutoff point was selected for the detection of significant upregulation or downregulation of the genes in the mRNA amount between the arrays. Using the signal intensity of selected genes that were up or downregulated compared with the sham–VMH-lesioned control group, analysis was performed using GeneSpring GX version 10 (Agilent Technologies, Santa Clara, CA).

2.5. Real-time PCR analysis

Real-time PCR analysis was performed as described previously [7]. The RNAs of three individual rats at day 3 after VMH lesioning and three sham–VMH-lesioned rats were stored at −70 °C until this analysis was performed. Relative quantification of gene expression with real-time PCR data was calculated relative to glyceraldehyde 3-phosphate dehydrogenase. In the present study, two representative genes related to metabolisms were investigated by real-time PCR: (1) arachidonate 15-lipoxygenase (Alox15) and (2) pancreatic lipase (Pnlip). Real-time PCR primers were inventoried in the TaqMan gene expression assay, for Aloxl5 (Rn00696151_m1) or Pnlip (Rn00565851_m1) and GADPH (Rn01775763_g1) (Applied Biosystems).

2.6. Data analysis

Results were expressed as mean ± SEM. The mRNA levels were analyzed by the Mann–Whitney *U*-test. Statistical analysis was conducted with SPSS version 19.0 statistical software (SPSS Japan Inc., Tokyo, Japan). The differences between the groups were considered significant if $p < 0.05$ (2-tailed).

We analyzed differential expressed gene lists with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) functional annotation web tool (http://david.abcc.ncifcrf.gov/) and obtained lists of enriched KEGG pathways. In this study the differentially expressed genes in the network underwent functional enrichment analysis by DAVID. A $p < 0.05$ was set as the threshold.

3. Results

Among 17,061 probes, the expression of 758 probes (4.4%) showed at least a 2-fold upregulation (230 probes) or downregulation (528 probes) at day 3 after VMH lesioning as compared with sham–VMH lesioning. Supplementary Table 1 shows the upregulated (>2-fold) and the downregulated (>2-fold) metabolism-related, identified by DNA microarray analysis. The results revealed that VMH lesions regulated some genes that are involved in enzyme (upregulated genes 7 probes; downregulated genes 35 probes), hormone (upregulated genes 6 probes; downregulated genes 1 probes), peptides (downregulated genes 14 probes), and chemical compounds (downregulated genes 1 probes). Regarding the results of real-time PCR, the gene expressions of Aloxl5 was upregulated and that of Pnlip was downregulated ($p < 0.05$; Fig. 1).

The results revealed VMH lesions upregulated some genes that are involved in hormone activity (Table 1), and downregulated some genes that are involved in response to steroid hormone stimulus, response to hormone stimulus, response to nutrient, digestion and behavior (Table 1).

DAVID functional annotation analysis revealed that the molecular pathway enriched in upregulated genes families included that associated with arachidonic acid pathway (Table 2), whereas the molecular pathways in downregulated genes families included type 1 diabetes mellitus, renin–angiotensin system and linoleic acid metabolism (Table 2).