マウスにおける咀嚼動態および咀嚼習慣の相違がGLP-1分泌に与える影響

Abstract

The Effect of Mastication by Food Forms on The Secretion of GLP-1 in Mice

2016

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【Objective】

The number of patients with diabetes has recently increased rapidly in Japan. Diabetes lowers patient quality of life as a result of complications of the disease. As such, diabetes prevention is great importance.

Glucose tolerance is affected in Type 2 diabetes. Glucose tolerance develops as a result of both environmental and genetic factors, with lifestyle being a key environmental factor. Eating habits are related to lifestyle, and include caloric intake and nutritional quality, both of which are factors in diabetes. Mastication is an important part of an individual's eating habits.

Mastication involves the mechanical crushing and grinding of food with the teeth into small particles that enable the gastrointestinal absorption of nutrients. It has been reported that mastication indirectly activates the parasympathetic nerve and promotes gastrointestinal hormone secretion.

Glucagon-like peptide-1 (GLP-1) is one of gastrointestinal hormone and synthesized in the enteroendocrine L cells of the intestine. GLP-1 employs the incretin effect to lower levels of glucose in the blood and provide ß-cell protection. The secretion of GLP-1 is regulated by the vagus nerve and the mechanical stimulus from digested food.

This study hypothesizes that the mastication of solid food promotes GLP-1 secretion via vagus nerve. I examined how mastication affects the secretion of GLP-1, the protection of pancreatic ß-cells, and the secretion of insulin.

【Materials and methods】

This experimental protocol was approved by the animal ethics and research committee of the Health Sciences University of Hokkaido. 190

Male C57BL/6J mice were obtained for use in this experience. I examined 2 experiments. In experiment 1, I analyzed the masticatory affect on the blood glucose level, the secretion of GLP-1 and insulin. In experiment 2, I analyzed masticatory habit affect on the secretion of GLP-1, insulin, and cross-section area of ß-cells.

In experiment 1, Sixteen-week-old mice were starved for 24 h and divided into two groups: a solid feed group (n=10) and a liquid feed group (n=10).A solid feed group and a liquid feed group (n=10) were fed solid or liquid feed at 5 min. The blood glucose levels were measured using blood glucose measurement kit (Nipro freestyle freedom light, Nipro). Blood from the heart was collected in a tube containing dipeptidyl peptidase-4 inhibitor (which cleavages and inactivates GLP-1) at 30, 60, 90 and 120 min after the start of feeding. The active GLP-1 concentration was measured using a GLP-1 active form assay kit (Immuno-biological Laboratories). Insulin concentration was measured using an Ultrasensitive Mouse Insulin ELISA Kit (Morinaga Institute of Biological Science).

Next, mice with atropine (atropine solid feed group and atropine liquid feed group, n=10), mice with fed 0 kcal feed (0 kcal solid feed group and 0 kcal liquid feed group, n=10), and 4weeks mice (4-week-old solid feed group and 4-week-old liquid feed group, n=10) were checked the blood glucose levels, active GLP-1 concentration, and insulin concentration at 30 min in the same manner.

In experiment 2, 4-week-old mice were divided into two groups: a long-term solid feed group and a long-term liquid feed group. Long-term solid feed group and long-term liquid feed group were raised for an additional 12 weeks. Body weight and blood glucose levels were checked every week. At 16 weeks, mice were starved for 24 h, and underwent an

oral glucose tolerance test (OGTT). Also, at 4 and 16 weeks mice were deeply anaesthetized with 4% isoflurane. Blood was collected from the heart, and blood glucose level, active GLP-1 concentration, and insulin concentration were measured in the same manner. All blood data were measured at the same time.

The pancreas was enucleated at 4 and 16 weeks. Pancreases were fixed by 4% formaldehyde. Immunostaining of paraffin-embedded sections using (5 μm), guinea pig anti insulin antibody was used for the primary antibody, and goat anti rabbit Ig-fab-peroxidase conjugate was used for the secondary antibody, before staining by DAB. The sections were counterstained with methyl green for identification. The stained pancreatic tissue mounted on slides was analyzed by Image J software.

The data were compared by the Mann-Whitney's U-test and were compared the 4 groups by Kruskall Wallis test followed by Mann-Whitney's U test and Bonferroni correction. Comparison of the changes of blood glucose level, active insulin concentration, and insulin concentration was compared by ANOVA followed by Tukey test. In statistical analyses, $p < 0.05$ was considered statistically significant.

【Results】

In experiment 1, the blood glucose levels in the solid feed group and the liquid feed group were higher at 30, 60, and 90 min than at 0 min and approximately equal at all times (ANOVA, $p < 0.05$). At 30 and 90 min, the active GLP-1 concentration in the solid feed group was higher than that in O min (ANOVA, $p < 0.05$).And at 30 min, the active GLP-1 concentration in the solid feed group was higher than that in the liquid feed group (MWU, $p < 0.05$) . In mice with atropine, blood glucose level in atropine solid feed group and atropine liquid feed group was lower than

that in solid feed group and liquid feed group, but almost equivalent (Kruskall Wallis MWU, $p < 0.05$). And the active GLP-1 concentration in nerve blocked solid feed group and nerve blocked liquid feed group was lower than that in the solid feed group, but almost equivalent (Kruskall Wallis MWU, $p < 0.05$). In mice fed 0 kcal/kg, blood glucose levels in both groups were almost equivalent. And the active GLP-1 concentration in 0 kcal solid feed group and 0 kcal liquid feed group was lower than that in the solid feed group , but almost equivalent (Kruskall Wallis MWU, $p < 0.05$). And the active GLP-1 concentration in the 4-week-old solid feed group was higher than that in the 4-week-old liquid feed group.

Insulin concentrations were approximately equal in all cases.

In experiment 2, blood glucose levels, body weight, and oral glucose tolerance test results in the long-term solid feed group and the long-term liquid feed group were approximately equal. At 16 weeks, the active GLP-1 concentration in the long-term solid feed group was higher than that in the long-term liquid feed group and that in four weeks (MWU, $p < 0.05$), and the insulin concentration in the long-term solid feed group was higher than that in the long-term liquid feed group (MWU, $p < 0.05$) and that in four weeks (MWU, $p < 0.05$). And cross-section area of ß-cells in both groups increased for 12 weeks (MWU, $p < 0.05$), and the area of the long-term solid feed group was higher than that of the long-term liquid feed group (MWU, $p < 0.05$).

【Discussion】

Mastication affects the secretion of GLP-1 at 30 min. The active GLP-1 concentration at 30 min disappeared in both groups by blocking of the vagus nerve stimulation and by the absorption of nutrient in the intestine. Therefore, it can be said that the secretion of GLP-1 by

mastication is promoted by vagus nerve stimulation and by the absorption of nutrient in the intestine.

Masticatory habits affect not only active GLP-1 concentration but also increasing cross-section area of ß-cells and insulin concentration. This difference is believed to result from mastication, which promotes GLP-1, and increasing cross-section area of ß-cells by mastication affects insulin concentration.

The results of this study indicate that mastication affects GLP-1 and that masticatory habits affect insulin secretion. Mastication is known to prevent hyperphagia, and could therefore be considered to aid in the prevention of diabetes. Furthermore, mastication is also involved in glycometabolism through the secretion of GLP-1, and plays an important role in the prevention of glucose tolerance and diabetes.