YIL counteracts ghrelin-inhibited insulin release in pancreatic islets of Langerhans

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Abstract. Ghrelin is a peptide hormone that is produced mainly from the stomach. Ghrelin is reported to have many biological functions, such as modulating feeding behavior, energy balance, and glucose homeostasis. This study aimed to examine whether YIL, a ghrelin receptor antagonist, could counteract the effect of ghrelin-inhibited insulin release in the pancreatic islet of Langerhans. This study is experimental research using wild-type C57BL/6J mice [8-10 weeks old]. Islet of Langerhans was isolated by collagenase digestion and the insulin release [ng/islet/h] from the islet is examined by the ELISA method. Data represent means ± SEM and is analyzed by one-way ANOVA. The result showed that 8.3 mM glucose concentration increase insulin release compared to 2.8 mM glucose, respectively [0.393 ± 0.025 vs 0.219 ± 0.022 ng/islet/h]. In the presence of 8.3 mM glucose, ghrelin 1 nM showed a decrease in insulin release significantly compared to 8.3 mM glucose only [0.283 ± 0.001 vs 0.393 ± 0.025, p < 0.01]. In contrast, in the presence of 8.3 mM glucose and ghrelin 1 nM, YIL 1 µM induced insulin secretion [0.386 ± 0.012 vs 0.283 ± 0.001, p < 0.01]. In conclusion, YIL is significantly counteracted ghrelin-inhibited insulin release in pancreatic islets of Langerhans. Furthermore, YIL is one of the candidates for the treatment of type 2 diabetes.

Keywords: YIL, ghrelin-inhibited insulin release, pancreatic islets

1. Introduction

Ghrelin is a 28 amino acid peptide and endogenous ligand of growth hormone secretagogue receptor type 1a [GHSR1a] which is produced predominantly from the gastric.[1],[2] There are two types of circulating ghrelin in our body, des acyl ghrelin [90 %] and acyl ghrelin [10 %]. Desacyl ghrelin does not bind the GHSR1a, while acyl ghrelin binds the receptor and shows central and peripheral effects.[2] It has been reported that ghrelin has a wide range of biological functions, such as promoting food intake and appetite, regulating energy balance, increasing gastric acid secretion, and regulating glucose homeostasis [4],[5],[6].

It has been reported that both central and peripheral administration of ghrelin to rats induced food intake stimulation. In human, administration of ghrelin intravenously and subcutaneously enhance food intake.[7],[8] Fasting ghrelin levels in obese subjects lower than normal body weight and enhance in weight loss [3],[9],[10].

Ghrelin contributed to energy balance in the long term and influence the status of nutrition. Systemic ghrelin is adversely connected with body adiposity. Therefore, when ghrelin level increases,
prevention of ghrelin secretion may have therapeutic potential to enhancing further bodyweight loss.[4],[8] Ghrelin level is decreased by overfeeding, administration of glucocorticoid, and increasing body weight induced by a high-fat diet.[4],[11],[12] Furthermore, ghrelin levels enhanced by exercise, weight loss induced by a low-calorie diet, anorexia nervosa, or cachexia due to organ damage.[13],[14] Other studies reported that long-term ghrelin effects in rodents caused prolonged hyperphagia, increase body weight, activation of adipogenesis, inhibition of apoptosis, inhibit sympathetic nervous system activity, and decrease energy expenditure [4],[15],[16],[17].

Ghrelin expression in pancreatic islets of Langerhans has attracted researchers to examine the role of ghrelin in glucose homeostasis. Our group found that glucose-induced insulin release in isolated islets was attenuated by ghrelin at 10 nM, but basal insulin release at 2.8 mM glucose either in isolated islets or perfused pancreas was not affected by ghrelin administration.[18] This result indicated that ghrelin possibly plays an important role in regulating insulin release in pancreatic islets. A previous study in ob/ob mice with GHS-R1a deficiency showed an increase in insulin secretion and improving glucose tolerance. Furthermore, GHS-R antagonists may have an important role in the treatment of type 2 diabetes mice.[19] This study aims to examine the effect of YIL, a ghrelin receptor antagonist, on ghrelin-inhibited insulin release in islets of Langerhans.

2. Material and Methods

Animals
Wild-type C57BL/6J mice [male, 10-12 weeks old] were maintained following Jichi Medical University and the Japanese Physiological Society’s guidelines.

2.1. Islet Isolation Preparation
Collagenase digestion methods were used for islet isolation. Before isolation, pentobarbital at a dosage of 80 mg/kg was injected intraperitoneally into mice. Collagenase from Sigma-Aldrich [1.05 mg/ml] was dissolved in 5 mmol/l Ca2+ containing HEPES-added Krebs-Ringer bicarbonate buffer [HKRB] solution and then injected into the common bile duct. The HKRB solution consist of 129 mmol NaCl, 5 mmol/l NaHCO3, 4.7 mmol/l KCl, 1.2 mmol/l KH2PO4, 2 mmol/l CaCl2, 1.2 mmol/l MgSO4, and 10 mmol/l HEPES, at pH 7.4 with NaOH. HKRB was added with 0.1% BSA. The pancreas was dissected out and continue by incubation at 37°C for 16 minutes. Islets of Langerhans were collected and prepared for measurement of insulin release.

2.2. Measurements of insulin release in mouse islets
Groups of 10 islets were incubated for 1 hour at 37°C in HKRB with 2.8 mM glucose for stabilization, followed by test incubation for 1 hour in HKRB with 2.8 mM or 8.3 mM glucose. Ghrelin [Peptide Institute, Osaka, Japan] and YIL [Sigma-Aldrich] with ghrelin were present throughout the incubation. Insulin release in islets was determined by an ELISA kit [Morinaga Institute of Biological Science, Japan].

2.3. Statistical analysis
Data represent the means ± SEM. Statistical analyses were performed using one-way ANOVA and followed by Bonferroni multiple comparison tests, and p values below 0.05 were considered statistically significant.
3. Results

**YIL counteracts ghrelin-inhibited insulin release in isolated pancreatic islets**

![Figure 1](image)

**Fig. 1.** YIL counteracts ghrelin-inhibited insulin release in isolated islets of Langerhans. 8.3 mM glucose-induced insulin release in isolated islets. Ghrelin [1 nM]-inhibited insulin release at 8.3 mM glucose. YIL [1 µM] counteracts ghrelin-inhibited insulin release. \( n = 8-11 \) tubes. Ten islets in one tube were used for insulin measurement. **\( p < 0.01 \).**

4. Discussion

The present study demonstrated that YIL, a ghrelin receptor antagonist, blocked ghrelin-inhibited insulin release in islets of Langerhans. This result was similar to the previous study by the Esler group.[19] In this study, we used ghrelin 1 nM and showed a decrease in insulin release significantly. This concentration is lower than in the previous study [18].

In β-cells pancreas, ghrelin binds and activates growth hormone secretagogue receptor [GHS-R] that is coupled with pertussis toxin [PTX]-sensitive heterotrimeric G-protein Ga\( \alpha \)2 and then decreases cAMP production. This condition activates voltage-dependent Kv channels [Kv2.1 subtype] and decreases membrane excitability, and causing suppresses Ca\( ^{2+} \) influx and insulin release.[6]

Administration of ghrelin in mice, rats, and human-caused a decrease in insulin concentration after overnight fasting.[18],[20],[21] These results suggested that ghrelin plays an important role in regulating glucose homeostasis.

We found that YIL, a small molecule of ghrelin receptor antagonist, blocked ghrelin function in pancreatic islets of Langerhans. Blockade of ghrelin function in β-cells of pancreas consequently increases insulin release.

5. Conclusion

YIL counteracted ghrelin-restricted insulin release in isolated islets of Langerhans. In the future, it is important to examine the role of YIL in isolated islets of type 2 diabetic model mice.

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