Liver-Expressed Antimicrobial Peptide 2 antagonizes the insulinostatic effect of ghrelin in rat isolated pancreatic islets

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LEAP2 antagonizes the insulinostatic effect of ghrelin in rat isolated pancreatic islets

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Title: LEAP2 antagonizes the insulinostatic effect of ghrelin in rat isolated pancreatic islets

Running title: LEAP2 and insulin secretion

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**SUMMARY:**

**Background:** The hormone ghrelin is the endogenous agonist of the G-protein coupled receptor (GPCR) termed growth-hormone secretagogue receptor (GHSR). Ghrelin inhibits glucose-stimulated insulin secretion by activating pancreatic GHSR. Recently, Liver-Expressed Antimicrobial Peptide 2 (LEAP2) was recognized as an endogenous GHSR ligand that blocks ghrelin-induced actions. Nonetheless, the effect of LEAP2 on glucose-stimulated insulin secretion from pancreatic islets is unknown.

**Objectives:** We aimed at exploring the activity of LEAP2 on glucose-stimulated insulin secretion.

**Methods:** Islets of Langerhans isolated from rat pancreas were exposed to glucose in the presence or in the absence of LEAP2 and ghrelin and then insulin secretion was assayed.

**Results:** LEAP2 did not modulate glucose-stimulated insulin secretion. However, LEAP2 blocked the insulinostatic action of ghrelin.

**Conclusion:** Our data show that LEAP2 behaves as an antagonist of pancreatic GHSR.

**KEY WORDS:** LEAP2; ghrelin; GHS-R1a; insulin secretion; islets

**ABBREVIATIONS:** GHSR, growth-hormone secretagogue receptor; GPCR, G-protein coupled receptor; LEAP2, Liver-Expressed Antimicrobial Peptide 2

**MAIN TEXT:**

Ghrelin is a peptide hormone that operates through the binding and activation of the Gq-coupled GPCR named growth-hormone secretagogue receptor (GHSR). GHSR regulates various key physiological processes in particular the ones related to energy homeostasis including glucose metabolism [1,2]. Indeed, ghrelin administration induces blood glucose elevation and reduction in circulating insulin in humans and rodents [3]. Mechanistic studies undertaken in rodent isolated islets of Langerhans showed that ghrelin inhibits glucose-stimulated insulin secretion through the activation of the endogenously-expressed pancreatic GHSR [1,3,4].

Besides ghrelin, Liver-Expressed Antimicrobial Peptide 2 (LEAP2) was recently identified as a new endogenous GHSR ligand [5]. LEAP2 exhibits both antagonist and inverse agonist activities toward GHSR [5–7]. We previously reported that LEAP2 displays \( K_i \) value within the nanomolar range (1.26 ± 0.05 nM) and a \( pA_2 \) value of 7.99 ± 0.15 with respect to Gq-induced intracellular calcium mobilization [6]. Since then, a great deal of studies devoted to delineating LEAP2 function indicated that this peptide counteracts ghrelin-mediated actions including food intake and growth hormone secretion in rodent models [2,5,6,8]. Despite this rapid progress in characterizing LEAP2 activity, the effect of this peptide on glucose-stimulated insulin secretion is unknown. Here, we addressed this question using isolated islets of Langerhans since this biological system recapitulates the insulinostatic action of ghrelin [4,6,9]. All animal care and experiments complied with the directive 2010/63/EU and were approved by the local animal care and use committee Languedoc-Roussillon under reference CEEA-LR-19002.

Isolation, preparation and treatments of rat pancreatic islets as well as insulin assays were performed exactly as previously described [6,10]. Briefly, islets were incubated (120 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 24 mM NaHCO₃, and 0.1% (w/v) BSA, pH 7.4) in the presence or absence of LEAP2, ghrelin and glucose at the concentration indicated in the figure legend. After 1 hour of incubation at 37°C, the extracellular medium was collected and insulin concentration was quantified. Rat ghrelin (#1465) and rat LEAP2 (#075-50) were obtained from Tocris and Phoenix Pharmaceuticals Inc respectively.

In a first set of experiments, pancreatic islets were incubated with or without LEAP2 in the presence of non-stimulating (2.8 mM) or stimulating (8.3 mM and 16.6 mM) glucose concentrations (Figure 1A).

We used LEAP2 at 100 nM because this concentration was reported to be sufficient to fully displaced...
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ghrelin from GHSR in competition assays and to antagonize ghrelin effects in cellular models [6,7]. Figure 1A showed that LEAP2 did not alter glucose-stimulated insulin secretion regardless of glucose concentration. Next, a concentration–response relationship of LEAP2 (1; 10 and 100 nM) on insulin secretion was carried out under 8.3 mM-stimulating glucose condition (Figure 1B). Such an intermediate stimulating glucose concentration is appropriate to reveal a potential stimulatory or inhibitory effect of a compound on insulin secretion [10]. Figure 1B showed that LEAP2 was unable to modulate glucose-stimulated insulin secretion regardless of its concentration. Finally, we tested whether LEAP2 could affect the insulinstatic action of ghrelin. Therefore, insulin release was quantified following the incubation of pancreatic islets with 8.3 mM glucose in the presence or absence of 10 nM ghrelin and 100 nM LEAP2 (Figure 1C). As expected, ghrelin decreased glucose-stimulated insulin secretion. Importantly, concomitant incubation of pancreatic islets with both ghrelin and LEAP2 fully blocked the inhibitory effect of ghrelin on insulin secretion. These data showed that LEAP2 binds to pancreatic GHSR and behaves as an antagonist by blocking its activation by ghrelin. Our findings are reminiscent of recent studies demonstrating that LEAP2 suppresses ghrelin-mediated effects [5,6,8] and further expand the pharmacological characterization of LEAP2 at level of pancreatic endocrine function.

REFERENCES:

**FIGURE LEGEND:**

Figure 1: Effect of LEAP2 on insulin secretion in rat isolated pancreatic islets. Rat pancreatic islets were incubated (A) under 2.8 mM glucose, 8.3 mM or 16.6 mM glucose-stimulated conditions in the presence or absence of LEAP2 (100 nM); (B) under 2.8 mM glucose or 8.3 mM glucose-stimulated conditions in the presence of different concentrations of LEAP2; (C) under basal (2.8 mM glucose) or 8.3 mM glucose-stimulated conditions in the presence or absence of ghrelin (10 nM) and LEAP2 (100 nM). The histogram depicts the mean ± SEM of insulin secretion expressed in ng / mL / islet. In (A), two-way ANOVA showed an effect of glucose \( [P < 0.0001] \), no effect of LEAP2 and no interaction between both factors. In (B), one-way ANOVA performed with 8.3 mM glucose data points showed no effect of LEAP2. In (C), two-way ANOVA performed with 8.3 mM glucose data points showed an interaction between ghrelin and LEAP2 \( [P < 0.0001] \). ns \( p \geq 0.05 \); **** \( p < 0.0001 \), Holm-Sidak multiple comparison test. \( n = 4 \) per group.