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

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

2 **Running title:** LEAP2 and insulin secretion

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15

16 **SUMMARY:**

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

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

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

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

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

29

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

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

33 **MAIN TEXT:**

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

41 Besides ghrelin, Liver-Expressed Antimicrobial Peptide 2 (LEAP2) was recently identified as a new
42 endogenous GHSR ligand [5]. LEAP2 exhibits both antagonist and inverse agonist activities toward
43 GHSR [5–7]. We previously reported that LEAP2 displays K_i value within the nanomolar range (1.26
44 ± 0.05 nM) and a pA_2 value of 7.99 ± 0.15 with respect to Gq-induced intracellular calcium
45 mobilization [6]. Since then, a great deal of studies devoted to delineating LEAP2 function indicated
46 that this peptide counteracts ghrelin-mediated actions including food intake and growth hormone
47 secretion in rodent models [2,5,6,8]. Despite this rapid progress in characterizing LEAP2 activity, the
48 effect of this peptide on glucose-stimulated insulin secretion is unknown. Here, we addressed this
49 question using isolated islets of Langerhans since this biological system recapitulates the insulinostatic
50 action of ghrelin [4,6,9].

51 All animal care and experiments complied with the directive 2010/63/EU and were approved by the
52 local animal care and use committee Languedoc-Roussillon under reference CEEA-LR-19002.
53 Isolation, preparation and treatments of rat pancreatic islets as well as insulin assays were performed
54 exactly as previously described [6,10]. Briefly, islets were incubated (120 mM NaCl, 4.7 mM KCl, 1.2
55 mM KH_2PO_4 , 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 24 mM $NaHCO_3$, and 0.1% (w/v) BSA, pH 7.4) in the
56 presence or absence of LEAP2, ghrelin and glucose at the concentration indicated in the figure legend.
57 After 1 hour of incubation at 37°C, the extracellular medium was collected and insulin concentration
58 was quantified. Rat ghrelin (#1465) and rat LEAP2 (#075-50) were obtained from *Tocris* and *Phoenix*
59 *Pharmaceuticals Inc* respectively.

60 In a first set of experiments, pancreatic islets were incubated with or without LEAP2 in the presence of
61 non-stimulating (2.8 mM) or stimulating (8.3 mM and 16.6 mM) glucose concentrations (**Figure 1A**).
62 We used LEAP2 at 100 nM because this concentration was reported to be sufficient to fully displaced
63 ghrelin from GHSR in competition assays and to antagonize ghrelin effects in cellular models [6,7].
64 **Figure 1A** showed that LEAP2 did not alter glucose-stimulated insulin secretion regardless of glucose
65 concentration. Next, a concentration–response relationship of LEAP2 (1; 10 and 100 nM) on insulin
66 secretion was carried out under 8.3 mM-stimulating glucose condition (**Figure 1B**). Such an
67 intermediate stimulating glucose concentration is appropriate to reveal a potential stimulatory or
68 inhibitory effect of a compound on insulin secretion [10]. In addition, circulating LEAP2
69 concentrations were reported to fluctuate in the nanomolar range [2,5,11]. **Figure 1B** showed that
70 LEAP2 was unable to modulate glucose-stimulated insulin secretion regardless of its concentration.
71 Finally, we tested whether LEAP2 could affect the insulinostatic action of ghrelin. To do so, we used a
72 tenfold higher concentration of LEAP2 with respect to ghrelin to mimic the ghrelin/LEAP2 circulating
73 molar ratio that was observed in satiated mice [2,11]. Therefore, insulin release was quantified
74 following the incubation of pancreatic islets with 8.3 mM glucose in the presence or absence of 10 nM
75 ghrelin and 100 nM LEAP2 (**Figure 1C**). As expected, ghrelin decreased glucose-stimulated insulin
76 secretion. Importantly, concomitant incubation of pancreatic islets with both ghrelin and LEAP2 fully
77 blocked the inhibitory effect of ghrelin on insulin secretion. These data suggested that LEAP2 binds to
78 pancreatic GHSR and behaves as an antagonist by blocking its activation by ghrelin. Our findings are
79 reminiscent of recent studies demonstrating that LEAP2 suppresses ghrelin-mediated effects [5,6,8]
80 and further expand the pharmacological characterization of LEAP2 at level of pancreatic endocrine
81 function.

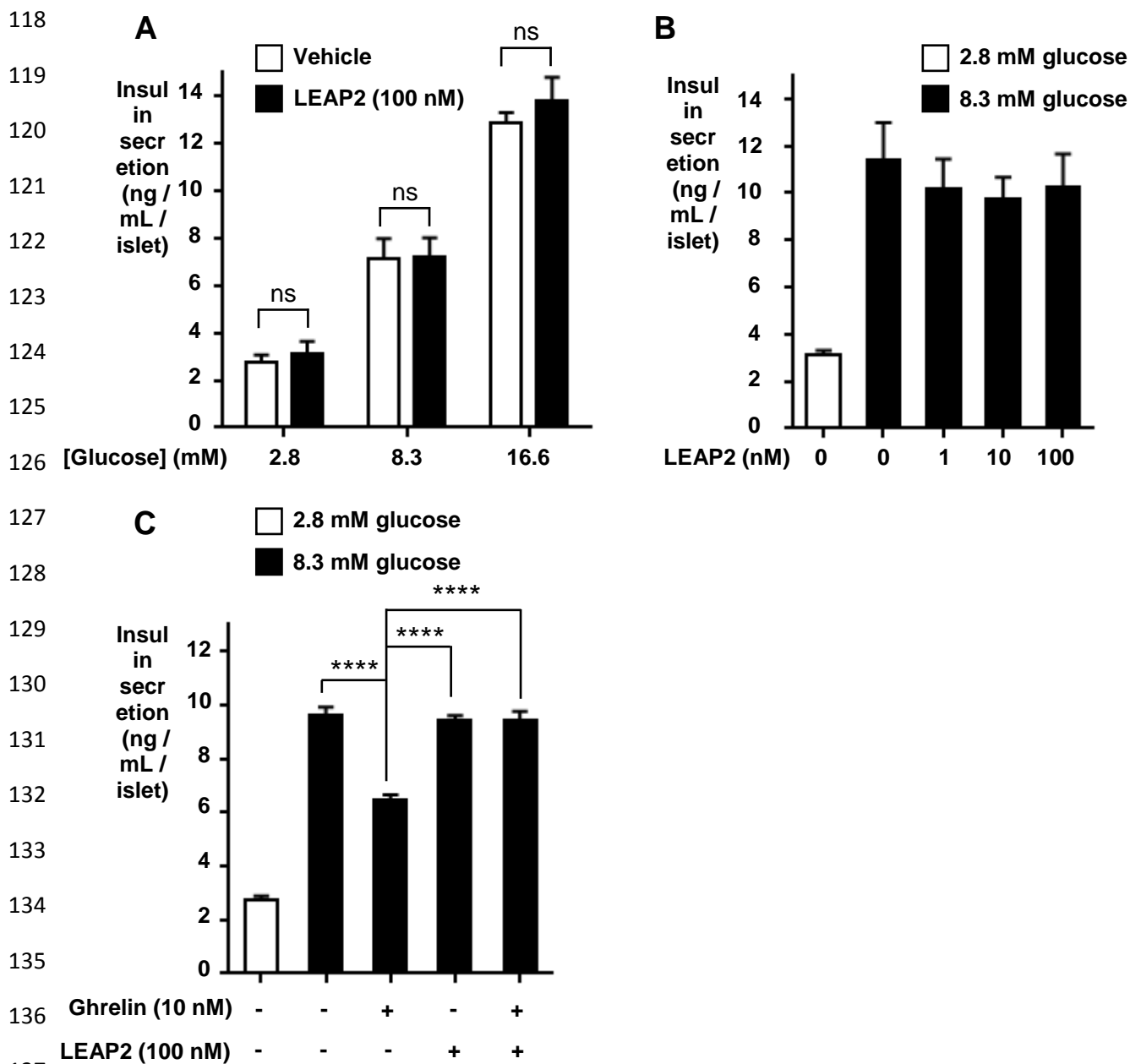
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 116

117 **FIGURE 1:**



138 **FIGURE LEGEND:**

139 **Figure 1: Effect of LEAP2 on insulin secretion in rat isolated pancreatic islets.** Rat pancreatic
 140 islets were incubated (A) under 2.8 mM glucose, 8.3 mM or 16.6 mM glucose-stimulated conditions in
 141 the presence or absence of LEAP2 (100 nM); (B) under 2.8 mM glucose or 8.3 mM glucose-
 142 stimulated conditions in the presence of different concentrations of LEAP2; (C) under basal (2.8 mM

143 glucose) or 8.3 mM glucose-stimulated conditions in the presence or absence of ghrelin (10 nM) and
144 LEAP2 (100 nM). The histogram depicts the mean \pm SEM of insulin secretion expressed in ng / mL /
145 islet. In (A), two-way ANOVA showed an effect of glucose [$P < 0.0001$], no effect of LEAP2 and no
146 interaction between both factors. In (B), one-way ANOVA performed with 8.3 mM glucose data
147 points showed no effect of LEAP2. In (C), two-way ANOVA performed with 8.3 mM glucose data
148 points showed an interaction between ghrelin and LEAP2 [$P < 0.0001$]. ns $p \geq 0,05$; **** $p < 0.0001$,
149 Holm-Sidak multiple comparison test. n = 4 per group.