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GHSR MODULATION BY LEAP2 IN PERINATAL PHASES IMPACTS GLUCOSE OVERLOAD RESPONSIVENESS IN YOUNG RATS

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Abstract

GHSr signaling is involved in the regulation of energy homeostasis. LEAP2, a recently discovered peptide hormone, counteracts the effects of ghrelin. This study sought to evaluate the effects of perinatal LEAP2 exposure in young rat offspring. For this, two protocols were designed to assess the roles of GHSr antagonism during the pregnancy and during the lactation. Sex-specific impacts on AUC and peak-responses of glucose tolerance tests were presented. However, no differences were detected in the blood glucose of mixed meal fed animals. In conclusion, perinatal LEAP2 exposure could impact the glucose-stimulated insulin secretion in young rats.

Keywords: GHSr Signalling, DOHaD, Glucose Homeostasis

1. Introduction

It is known that ghrelin has actions beyond the control of eating behavior, being important for the development and exerting effects in organs and systems, including in the endocrine pancreas (1). LEAP2, a recently discovered endogenous hepatic peptide with antagonistic actions towards GHSr, has been shown to counter the effects of ghrelin on glucose homeostasis (2,3). It is also known that changes in signaling pathways during perinatal phases can remain for a long time, as an effect of metabolic programming.

Therefore, the objective of this study was to evaluate the effects of perinatal exposure to LEAP2 in the young offspring of Wistar rats.

2. Material and methods

Protocol 1: Pregnant Wistar rats were separated into two groups: VEH, injected with vehicle (PBS) (n=5); and LEAP2, injected with 72 nmol/kg LEAP2(1-14-NH_2) (n=5). The injections were performed in the second half of pregnancy from GD10 to GD20.

Protocol 2: Pregnant Wistar rats (n=14) was assigned to this protocol. At PND3, the litter was adjusted to 8 pups per litter (4 of each sex). The pups of each litter were assigned to the following groups: VEH, injected with vehicle (PBS); LEAP2 (0.1) and LEAP2 (1), injected respectively with 0.1 or 1 μmol/kg LEAP2. The injections were done from PND3 to PND14, early in the morning.

Animals of both groups were allowed to grow up until PND45, when a glucose tolerance test (GTT) was performed in 6-hour fasted animals. The GTT was consisted by an oral administration of 2 g/kg glucose overload. For the measurement of blood glucose, blood samples were collected before, and 15, 30, 60 and 120 minutes after glucose administration. The results were expressed in mg/dL.

Two days after GTT, under 6h fasting, the animals of both groups were separated in two subgroups: SF, animals euthanized after 6h fasting; and PP, SF animals which received oral mixed meal. Mixed meal was composed by 59% carbohydrate, 11% protein, 25% fat (1.5 Kcal/animal). Before anesthesia, blood glucose of all animals was measured. PP animals were euthanized 1h after mixed meal administration. The pancreas was weighed and fixed to perform histological analysis. Hematoxylin-eosin stained slides were analyzed to assess pancreatic islets area and the results were expressed in μm^2.

Data are expressed as Mean ± SEM. To compare the effects of LEAP2 administration during pregnancy or lactation in time-dependent parameters, a two-way ANOVA was used followed by the Sidak post-test. For time-independent comparisons, Student's T test was used for comparisons between groups in Protocol 1, and a one-way ANOVA was used for comparisons between groups in the lactation protocol (post-Tukey test). The significance level was set at $p<0.05$.

3. Results and discussion

Intrauterine exposure to LEAP2 was not able to change peak-response to oral administration of glucose in glycemia and neither the response of the mixed meal intake, except for reducing AUC of GTT in female offspring of LEAP2 injected mothers (VEH 7260 ± 325.9 vs LEAP2 6249 ± 208.7 a.u.). In LEAP2 female offspring, the lower response to oral glucose administration is evidenced at 60 minutes of GTT ($p<0.01$ vs VEH). Intrauterine exposure to LEAP2 was not also able to alter the morphology of pancreatic islets in the offspring, despite higher means of area of pancreatic islets in males and females, and displacement of the greater relative frequency of islets from larger area in males. The histogram of pancreatic islet area in females remained similar to that of the control group.

The modulation of GHSr signaling through the administration of LEAP2 during lactation was not able to cause changes in glucose dynamics. In this sense, LEAP2 early exposure was able to significantly alter the glucose dynamics during the GTT in males in the first 15 min ($p<0.01$; VEH 8241 ± 572,5 vs LEAP2(0.1) 7287 ± 338.5 and LEAP2(1) 7389 ± 235.7 a.u.). There were no alterations in the glucose dynamics in females.

Studies that do not involve metabolic programming shown that under ghrelin regulation, the glucose-stimulated insulin secretion were reduced (1,4,5). However, when GHSr was blocked by [D-Lys3]-GHRP-6, blood glucose was remarkably decreased as a result of increased insulin release (5). In this sense, LEAP2 expression were correlated with improved insulin secretion in overweight humans (3), and antagonized ghrelin actions in isolated pancreatic islets (2).

4. Conclusion

Our results demonstrate that LEAP2 exposure in perinatal phases could impact in the glucose homeostasis. We hypothesized that perinatal antagonism of GHSr promoted a long-term inhibition of ghrelin signaling, resulting in increased glucose-stimulated insulin secretion. However, more experiments are needed to state that these results may not be due to greater insulin sensitivity.

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References

1. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, et al. Ghrelin, a Natural GH Secretagogue Produced by the Stomach, Induces Hyperglycemia and Reduces Insulin Secretion in Humans. J Clin Endocrinol Metab [Internet]. 2001 Oct;86(10):5083–5083. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.86.10.8098
2. Bayle M, Péraldi-Roux S, Gautheron G, Cros G, Oiry C, Neasta J. Liver-Expressed Antimicrobial Peptide 2 antagonizes the insulinostatic effect of ghrelin in rat isolated pancreatic islets. Fundam Clin Pharmacol [Internet]. 2022 Apr 8;36(2):375–7. Available from: https://onlinelibrary.wiley.com/doi/10.1111/fcp.12722
3. Stark R, Feehan J, Mousa A, Andrews ZB, de Courten B. Liver-expressed antimicrobial peptide 2 is associated with improved pancreatic insulin secretion in adults with overweight and obesity. Diabetes, Obes Metab [Internet]. 2023 May 19;25(5):1213–20. Available from: https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.14968
4. Dezaki K, Kakei M, Yada T. Ghrelin Uses Gαi2 and Activates Voltage-Dependent K+ Channels to Attenuate Glucose-Induced Ca2+ Signaling and Insulin Release in Islet β-Cells. Diabetes [Internet]. 2007 Sep 1;56(9):2319–27. Available from: https://diabetesjournals.org/diabetes/article/56/9/2319/12703/Ghrelin-Uses-G-i2-and-Activates-Voltage-Dependent
5. Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, et al. Endogenous Ghrelin in Pancreatic Islets Restricts Insulin Release by Attenuating Ca2+ Signaling in β-Cells. Diabetes [Internet]. 2004 Dec 1;53(12):3142–51. Available from: https://diabetesjournals.org/diabetes/article/53/12/3142/14492/Endogenous-Ghrelin-in-Pancreatic-Islets-Restricts