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Lacking of estradiol reduces insulin exocytosis from pancreatic β-cells and increases hepatic insulin degradation

Roberta S. Santos [a,b,*], Thiago M. Batista [a], Rafael L. Camargo [a], Priscila N. Morato [a], Patrícia C. Borck [a], Nayara C. Leite [a], Mirian A. Kurauti [a], Amarylis C.B.A. Wanschel [a], Ángel Nadal [c], Deborah J. Clegg [b], Everardo M. Carneiro [a]

[a] Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, e Centro de Pesquisa em Obesidade e Comorbidades, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil
[b] Biomedical Research Department, Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Beverly Hills, CA, United States
[c] CIBER de Diabetes y Enfermedades Metabólicas Asociadas, CIBERDEM, Universidad Miguel Hernández de Elche, Elche, Alicante, Spain

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ABSTRACT

Low levels of plasma estrogens are associated with weight-gain, android fat distribution, and a high prevalence of obesity-related comorbidities such as glucose intolerance and type II diabetes. The mechanisms underlying the association between low levels of estrogens and impaired glucose homeostasis are not completely understood. To begin to test this, we used three-month-old female C57BL/6J mice that either underwent ovariectomy (OVX) or received a sham surgery (Sham), and we characterized glucose homeostasis. In a subsequent series of experiments, OVX mice received estradiol treatment (OVX + E_2) or vehicle (OVX) for 6 consecutive days. As has been previously reported, lack of ovarian hormones resulted in dysregulated glucose homeostasis. To begin to explore the mechanisms by which this occurs, we characterized the impact of estrogens on insulin secretion and degradation in these mice. Insulin secretion and plasma insulin levels were lower in OVX mice. OVX mice had lower levels of pancreatic Syntaxin 1-A (Synt-1A) protein, which is involved in insulin extrusion from the pancreas. In the liver, OVX mice had higher levels of insulin-degrading enzyme (IDE) and this was associated with higher insulin clearance. Estradiol treatment improved glucose intolerance in OVX mice and restored insulin secretion, as well as normalized the protein content of pancreatic Synt-1A. The addition of estrogens to OVX mice reduced IDE protein to that of Sham mice. Our data suggest loss of ovarian estradiol following OVX led to impaired glucose homeostasis due to pancreatic β-cell dysfunction in the exocytosis of insulin, and upregulation of hepatic IDE protein content resulting in lower insulinemia, which was normalized by estradiol replacement.

1. Introduction

Low levels of plasma estrogens, which occur following menopause in women and ovariectomy (OVX) surgery in animal models, are associated with weight gain, increased amounts of visceral fat, and high prevalence of obesity-related comorbidities such as glucose intolerance, type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVDs) [1,2]. Women with higher estradiol levels, as seen in pre-menopausal women, have a lower prevalence of glucose intolerance when compared with age and weight matched men. The beneficial effects of estrogens on glucose homeostasis are reduced following menopause, when endogenous estrogen levels are low [3]. Previous reports have determined there is impaired glucose homeostasis following OVX-induced reductions in ovarian hormones, and this is associated with insulin resistance in animal models [1,4,5]. However, the mechanisms by which estrogens are involved in glucose homeostasis are not completely understood, and few studies have evaluated the effects of estradiol on insulin secretion and degradation.

Insulinemia is determined by pancreatic insulin secretion and hepatic insulin degradation. In pancreatic β-cells, insulin is released in response to increased blood glucose levels. Insulin production and packaging into granules, ATP production, closure of K_{ATP} channels, membrane depolarization, calcium influx, and insulin release involving the SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) proteins are all

* Corresponding author at: Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, UNICAMP, 255 Monteiro Lobato St, Block J, Campinas, SP 13083-862, Brazil.
E-mail address: santosrds1@gmail.com (R.S. Santos).

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required for the production and exocytosis of insulin. Specifically, the insulin exocytosis processes is initiated with an influx of calcium, followed by physical translocation and docking of the granules containing insulin to plasma membrane cholesterol-rich areas, and this is facilitated by the SNARE proteins such as Syntaxin-1 (Synt-1) and Synaptosomal-associated protein 25 (Snap-25) [6]. Estrogens mediate pancreatic insulin production and secretion primarily through two estrogens receptors, namely ERα (ERS1) and ERβ (ERS2). Recent evidence suggests ERα protects cells from oxidative stress-induced apoptosis, which is related to insulin production and secretion after a long-term exposure to glucose, while ERβ is involved in the functional regulation of membrane K_{ATP} channels [7], demonstrating two ways in which estrogens modulate insulin secretion. However, to our knowledge, no studies have sought to evaluate if there is a specific effect of estrogens also on the SNARE complex.

While insulin production and action are important, degradation, which mainly occurs in the liver through the insulin-degrading enzyme (IDE), also influences glucose homeostasis. In fact, alterations in the insulin-degrading process occur in some clinical conditions such as T2DM and obesity [8]. IDE is considered the primary enzymatic mechanism for cellular insulin degradation, which is a complex and multicomponent process. Since estradiol has an important impact on glucose, and the lack of estradiol is related to impaired glucose tolerance and insulin resistance, we hypothesized that estradiol also modulates hepatic insulin clearance and degradation. Thus, in the present study, we investigated the mechanisms underlying the relationship between lack of ovarian estrogens and impaired glucose homeostasis, specifically focusing on pancreatic β-cell insulin secretion and hepatic insulin degradation in OVX mice.

2. Materials and methods

2.1. Animals and experimental design

Four-week-old female C57BL/6J mice were purchased from the breeding colony at UNICAMP. For the first 90 days of life, mice were fed with a standard control diet (14% of calories from protein, 4% calories from fat) [9,10]. Mice then underwent bilateral OVX or sham procedure (Sham) [11]. In a second series of experiments, during the 14th week after surgery, OVX mice received subcutaneous estradiol treatment (β-E_2-3-benzoate, Sigma 46552) (OVX + E_2) or vehicle (mineral oil) (OVX) for 6 consecutive days at a dose of 50 μg/kg/day [12]. All described procedures were approved by the Ethical Committee of UNICAMP (Protocol 2999-1/2013).

2.2. Nutritional assessment

Body weight (BW) and food intake were measured 14 weeks following surgery. Mice were fasted and euthanized 14 weeks post-surgery. Commercial kits were used for analysis of plasma estradiol (Cayman Chemical Company, USA) and alanine aminotransferase (ALT) (Laborclin, Brazil). Additional samples of fresh liver and white adipose tissue were collected for histological sections according to Ribeiro et al. (2012) [13].

2.3. Energy expenditure and locomotor activity

During the 12–14 weeks after surgery, indirect calorimetry and spontaneous locomotor activity assessment were performed following a 24-h acclimation to the indirect calorimeter chambers. Oxygen consumption (VO_2) and CO_2 release (VCO_2) were measured for 24 h using the Oxylet® system (PanLab/Harvard Instruments, Barcelona, Spain). Locomotor activity was assessed by the Multitake Cage LE 001 PH system (PanLab/Harvard Instruments, Barcelona, Spain) throughout the entire 24 h.

2.4. Intraperitoneal glucose tolerance test (ipGTT) and intraperitoneal insulin tolerance test (ipITT)

IpGTT and ipITT were performed on the 14th week following the surgeries on different days [14]. For ipGTT, overnight (10 h) fasted mice were injected with a 20% glucose solution at a concentration of 2 g/kg body weight (BW). For ipITT, overnight fed mice (10 h) were fasted for two hours prior to the test, and mice were injected with 1 U/kg BW of human insulin (Biohulin® R, Biobrás, Montes Claros, MG, Brazil). The slope of glucose decay curve was calculated as previously described [15].

2.5. Insulin clearance, C-Peptide concentration and C-Peptide/insulin ratio

During ipGTT, plasma was collected before and after glucose injection to determine C-Peptide/insulin ratio. C-Peptide and insulin were determined using rat/mouse C-Peptide ELISA kit from Millipore (Cat. #EZRMCP2-21K; EZRMI-13K, Merck, KgaA©, respectively).

2.6. Islets isolation and static insulin secretion

Pancreatic islets were isolated by collagenase digestion of the pancreas, and incubated for 30 min in buffer supplemented with 5.6 mM glucose and 0.3% of bovine serum albumin (BSA), as previously described in Batista et al. (2012) [16]. Medium was then replaced with fresh buffer containing 2.8 or 16.7 mM glucose for 1 h. In another set of experiments, islets were incubated in a 2.8 mM glucose medium without NaCl and supplemented with or without 30 mM KCl. Insulin content from the medium was measured by RIA. For total islet insulin content, groups of four islets were collected from the 2.8 mM glucose condition.

2.7. Cytoplasmic Ca^{2+} oscillation

Cytoplasmic calcium oscillation was determined using fresh isolated pancreatic islets, as previously described by Batista et al. (2012) [16]. The islets were perfused with a BSA-free KBB containing 2.8 or 16.7 mM glucose.

2.8. Intracellular ATP production- Magnesium Green (MgG)

The fluorescence of the Magnesium Green probe (MgG, Molecular Probes, Life Technologies, Eugene, OR, USA) was measured in fresh isolated islets as an indirect quantification method for intracellular ATP, as previously described [17].

2.9. Western blot analysis

Protein expression was performed using the methods previously described by Batista et al. (2012) [16]. Membranes were incubated overnight with a polyclonal antibody against ERα (ab2746, Abcam) and IDE (ab32216, Abcam) for liver, and ERβ (ab16813, Abcam), Synt-1A (sc12736, Sta. Cruz), and Snap25 (s5187, Sigma-Aldrich) for pancreatic islets. Imaging and densitometry were performed using the Image Quant™ LAS 4000 Mini (GE® Healthcare Bio-Sciences) imaging system, and ImageJ software processing program.