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Fasting modulates GH/IGF-I axis and its regulatory systems in the mammary gland of female mice: Influence of endogenous cortistatin

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ABSTRACT

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are essential factors in mammary-gland (MG) development and are altered during fasting. However, no studies have investigated the alterations in the expression of GH/IGF-I and its regulatory systems (somatostatin/cortistatin and ghrelin) in MG during fasting. Therefore, this study was aimed at characterizing the regulation of GH/IGF-I/somatostatin/cortistatin/ghrelin-systems expression in MG of fasted female-mice (compared to fed-controls) and the influence of endogenous-cortistatin (using cortistatin-knockouts). Fasting decreased IGF-I while increased IGF-I/Insulin-receptors expression in MGs. Fasting provoked an increase in GH expression that might be associated to enhanced ghrelin-variants/ghrelin-O-acyl-transferase enzyme expression, while an upregulation of somatostatin-receptors was observed. However, cortistatin-knockouts mice showed a decrease in GH and somatostatin receptor-subtypes expression. Altogether, we demonstrate that GH/IGF-I, somatostatin/cortistatin and ghrelin systems expression is altered in MG during fasting, suggesting a relevant role in coordinating its response to metabolic stress, wherein endogenous cortistatin might be essential for an appropriate response.

1. Introduction

Growth hormone (GH) and insulin-like growth factor I (IGF-I) have been considered as essential factors in growth and development of the mammary gland (MG) (Kleinberg, 1997), especially since both hormones as well as their receptors (GH-R and IGF-IR) have been found to be locally produced at the MG (Botinaud et al., 2004; Mukhina et al., 2006; Yee et al., 1989). To a lesser extent, insulin is also known to participate in the physiology of MG through binding to its receptor (insulin receptor or IR) (Neville et al., 2013). Local expression of GH is crucial during puberty for cellular growth and differentiation, although is barely expressed in the MG of adult mice (Mukhina et al., 2006). For this reason, it has been suggested that circulating GH could be more relevant than locally produced GH in the development of MG under normal conditions (Cuttler et al., 2006). The main actions of local and circulating GH on MG, refer to stimulation of IGF-I production, which is pivotal for MG morphogenesis by induction of epithelial proliferation (Cannata et al., 2010). Indeed, in this case, local IGF-I fraction seems to be more important than circulating IGF-I in terms of controlling ductal development. In this scenario, several studies have proposed that changes in the GH/IGF-I axis of different tissues during starvation or fasting (an extreme metabolic state that alters the endocrine/metabolic homeostasis) may be the result of a metabolic adaptation to nutrient deprivation in several species, including mice and humans (Luque et al., 2007; Norrelund, 2005). Specifically, fasting is characterized by an elevation of circulating GH levels and a decreased free IGF-I bioavailability (Luque et al., 2011; Norrelund, 2005).

GH secretion from pituitary gland is primary regulated by the

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interplay of several inhibitory factors such as somatostatin (SST) and cortistatin (CORT) and, by stimulatory hormones such as ghrelin. Specifically, SST was discovered by its capacity to inhibit pituitary GH secretion (Brazeau et al., 1973); however, nowadays it is well known by its pleiotropic actions (Gahete et al., 2010a). Cortistatin (CORT) shows a remarkable structural, pharmacological and functional homology with SST (de Lecea et al., 1996), including their capacity to inhibit pituitary GH secretion (Broglio et al., 2008; Luque et al., 2006). However, recent studies have shown that CORT can also exert distinct actions from those exhibited by SST, in that CORT can differentially modulate immune system, inflammatory response or, even, the secretion of certain hormones (Cordoba-Chacon et al., 2011c; Gonzalez-Rey et al., 2006; Gonzalez-Rey and Delgado, 2008). This is also the case in the control of MG physiology, where CORT has been shown to be more potent than SST in the regulation of the local expression of GH/IGF-I axis in response to extreme conditions such as obesity (Villa-Osaba et al., 2015). Moreover, we have recently reported that CORT, rather than SST, could represent a major inhibitor of MG tumorigenesis under normal/lean-conditions and, that this regulation is exerted in a manner that is strongly dependent on the metabolic/endocrine milieu (lean vs. obese status) (Luque et al., 2016). To exert their functions, both SST and CORT bind with similar affinity to a family of receptors (sst1-5) with seven transmembrane domains (TMDs) coupled to G protein (Ben-Shlomo and Melmed, 2010), which are amply expressed through the organism (Patel, 1999) including the MG (Hatzoglou et al., 2000). In addition, it has been described the existence of a shorter truncated isoform of sst2, sst2B (Vanetti et al., 1992), and three functional truncated isoforms of sst5 in rodents, called sst5TMD4, sst5TMD2 and sst5TMD1, which are regulated at the pituitary level by changes in metabolic environment such as fasting conditions (Cordoba-Chacon et al., 2010).

On the other hand, ghrelin is an orexigenic peptide firstly identified from stomach extracts (Kojima et al., 1999; Shintani et al., 2001), which is also expressed in MG (Gronberg et al., 2008). Ghrelin can be acylated with an N-octanoic acid in the hydroxyl group of the third serine residue by the ghrelin-O-acyl transferase (GOAT) enzyme. This acylation is critical to appropriately bind its receptor, the growth hormone secretagogue receptor (GHS-R) (Gutierrez et al., 2008; Yang et al., 2008) and to exert its functions (including the stimulation of GH production). Previous studies have suggested that mouse ghrelin/GOAT system is regulated by energy status and metabolic signals, since stomach ghrelin expression is upregulated during fasting (Toshinai et al., 2001), suggesting that ghrelin could be involved in GH secretion during fasting (Muller et al., 2002). Furthermore, several studies have suggested that GOAT could have a biological relevance in the coordination of response to metabolic stress (fasting, obesity) in stomach, pituitary and hypothalamus (Gahete et al., 2010b). However, recent studies have shown that the complexity of the ghrelin system is higher than initially envisioned as ghrelin gene transcription can also generate different splicing variants, including the In2-ghrelin (Kineman et al., 2007), whose expression has been recently shown at the MG level (Villa-Osaba et al., 2015). Similar to ghrelin and GOAT, In2-ghrelin variant has been also shown to be regulated under extreme metabolic states in pituitary and hypothalamus (Kineman et al., 2007).

Despite the evidences suggesting that: 1) the endocrine axis comprised by GH/IGF-I (and likely its regulatory systems, SST/CORT, ghrelin and its receptors/associated enzymes) has an extraordinary relevance in MG physiology, 2) these systems are remarkably regulated in key metabolic tissues in various tissues in response to fasting and, 3) changes in the expression of these regulatory systems might be involved in the dysregulation of MG homeostasis in obese mice (Luque et al., 2016; Villa-Osaba et al., 2015); to the best of our knowledge, to date no studies have investigated if these systems might be altered in MGs under catabolic/fasting conditions, and whether endogenous CORT may exert a relevant role in the regulation of these systems in mice during fasting. Hence, in the present study, we hypothesized that, during fasting, a local deregulation in the GH/IGF-I axis (and/or its regulatory systems) may occur in an hormone-sensitive organ as is the MG which may condition the (patho)-physiology of the gland and, that the absence of endogenous CORT might be critical in some of the changes observed in these regulatory systems at the MG level under fasting conditions. Therefore, we aimed to characterize for the first time, the expression of GH/IGF-I, SST/CORT/ssts and ghrelin systems in MG of control (WT) and CORT-knockout (CORT-KO) female mice (C57Bl/6J) under fed conditions and during moderate and prolonged fasted conditions (24 and 48 h of fasting, respectively).

2. Materials and methods

2.1. Ethics statement

All experimental procedures for animal care and experimentation were approved by the IACUC of the University of Cordoba.

2.2. Animals and samples

Female C57Bl/6J wild-type (CORT+/+; Controls) mice were obtained by crossbreeding CORT ± mice and were housed (3–4 mice/cage) under standard conditions of light (12-h light, 12-h dark cycle; lights on at 07:00 h) and temperature (22–24 °C), with free access to tap water and food [standard rodent chow (SAFE-diets, Barcelona, Spain)]. At 8 weeks of age, mice were handled daily for two weeks to acclimate to personnel and handling methods. At 10 weeks of age, mice were randomly assigned to one of three groups (n = 7 mice/group): moderate (24 h) or prolonged (48 h) fasting (food removed at 8:00 a.m.) or *ad libitum* fed. During the fed or fasted period, all mice were placed in a solid floor using iso-PAD substrate (Omni BioResources, Inc; Cherry Hill, NJ, USA) to prevent any type of ingestion in the fasted groups.

In order to explore the consequences of the lack of endogenous CORT on the expression pattern of the MGs during the fasting state, a second group of female mice comprised by CORT-KO mice was used. CORT-KO mice (n = 6 mice/group) were generated by *in house* crossbreeding of an established colony of CORT ± and CORT −/− mice (Chanclón et al., 2013; Cordoba-Chacon et al., 2011c) and the same experimental protocol indicated above for female controls was followed.

Mice were weighed at the time of food withdrawal and just before death and, as previously reported (Luque et al., 2007; Norrelund, 2005), all mice were euthanized the same day by decapitation without anesthesia by authorized personnel. As previously described (Gahete et al., 2014a), trunk blood was collected and processed to obtain serum and plasma for hormone and metabolite determinations, whereas inguinal MGs were excised using sharp scissors, starting from the proximal area close to the nipple towards the distal end of the gland close to the spine of the animal, collecting all the adipose tissue delimiting the inguinal mammary area. Immediately after harvesting, MGs were frozen in liquid nitrogen and stored at −80 °C until their further processing. MGs constitute a heterogeneous population of cells that include adipose stroma and epithelial cells, whose proportions may be modified under different nutritional conditions. It should be noted that in the present study we focused the comparisons mainly between fed and fasted (24/48 h) groups within each genotype (WT or CORT-KO) since the initial main goal of the study was to determine, for the first time, the impact of fasting in the expression of GH/IGF-I,