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Active immunization against ghrelin decreases weight gain and alters plasma concentrations of growth hormone in growing pigs

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Abstract

Ghrelin has been implicated in the control of food intake and in the long-term regulation of body weight. We theorize that preventing the ability of ghrelin to interact with its receptors, would eventually lead to decreased appetite and thereby decrease body weight gain. To test our hypothesis, pigs were actively immunized against ghrelin. Ghrelin$_{(1–10)}$ was conjugated to BSA and emulsified in Freund's incomplete adjuvant and diethylaminoethyl-dextran. Primary immunization was given at 19 weeks of age (WOA), with booster immunizations given 20 and 40 days after primary immunization. Body weight (BW) and plasma samples were collected weekly beginning at 19 WOA, and feed intake was measured daily. Fourteen days after primary immunization, the percentage of bound ^{125}I-ghrelin in plasma from immunized pigs was increased compared with control animals ($P < 0.001$). Voluntary feed intake was decreased more than 15% in animals that were actively immunized against ghrelin compared with controls. By the end of the experiment, immunized pigs weighed 10% less than control animals ($P < 0.1$). Concentrations of GH were increased ($P < 0.05$) in immunized pigs. Apoptosis was not observed in post-mortem samples obtained from the fundic region of the stomach. Our observations suggest that immunization against ghrelin

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induces mild anorexia. This procedure could potentially be used as a treatment to control caloric intake and obesity.

Keywords: Ghrelin; Growth hormone; Immunization; Obesity; Pigs

1. Introduction

Ghrelin, a growth hormone-releasing peptide, was first isolated from rat stomach, while searching for an endogenous ligand to an "orphan" G-protein-coupled receptor [1]. The predominant active form of ghrelin is *n*-octanoylated ghrelin, a modification that has not been observed previously in mammalian physiology. Octanoylation is a post-translational process catalyzed by a still unidentified enzyme that attaches *n*-octanoic acid to amino residue Ser-3 to ghrelin. The *n*-octanoyl moiety is essential for the activation of ghrelin's growth hormone secretagogue receptor type 1a. In fact, non-acylated ghrelin (des-acyl ghrelin) impairs receptor action or activation [2,3]. Additionally, acylation of the peptide facilitates the passage across the blood–brain barrier. Although des-octanoyl mouse ghrelin cannot cross the blood–brain barrier in the brain-to-blood direction, human ghrelin can be transported in both directions in mice. These results suggest a complex, highly regulated bidirectional process, and a new role for the unique octanoyl component of ghrelin [4].

The octanoylated peptide is predominantly produced in the stomach within the oxyntic glands in rats and humans [5,6]. In fact, removal of the stomach in rats decreased serum ghrelin concentrations by 80%, suggesting that the stomach was the main source of ghrelin [5,7]. Lower amounts of ghrelin secretion and mRNA expression have been localized in several other tissues including other parts of the gut, the pituitary gland, and the hypothalamus [8,9].

Systemic exogenous administration of ghrelin induces adiposity in rodents by stimulating an acute increase in feed intake, as well as decreased fat utilization [10–12]. The orexigenic action of ghrelin is comparable to that of neuropeptide Y (NPY [13]). Unlike NPY, which is solely active when administered centrally (for review, see Ref. [14]), ghrelin induces feed intake when infused peripherally. Like leptin, but in an opposite manner, peripheral and central infusion of ghrelin influences both energy intake and metabolism. In fact, ghrelin is the first and only known peripheral orexigenic signal [13,15–17].

The metabolic and hormonal mechanisms by which nutrition signals the neuroendocrine axis are not known; however, it has been recognized that IGF-I and insulin are involved [18]. Ghrelin expression can be stimulated by hypoglycemia [19,20] and suppressed by hyperglycemia [21,22] and ingestion of sugar [10]. These data suggest that ghrelin integrates the hormonal and metabolic response to fasting with a negative feed back mechanism that might involve insulin and the activation of other mechanisms devoted to maintain glucose concentrations.

A pre-prandial increase in plasma ghrelin has been observed in humans [23], cattle [24,25], sheep [26], and rodents [10]. In rodents, peripheral or central administration of ghrelin increased feed intake, whereas passive immunization against ghrelin inhibited feed-