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Hexosamine biosynthetic pathway activity in leptin resistant sucrose-drinking rats

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HIGHLIGHTS

- Rats with access to sucrose solution are peripherally and centrally leptin resistant.
- Basal phosphorylated STAT3 is increased in the arcuate nucleus of the hypothalamus.
- Leptin does not further stimulate STAT3 phosphorylation in sucrose drinking rats.
- Glucosamine stimulates hypothalamic STAT3 phosphorylation.

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ABSTRACT

Rats offered 30% sucrose solution in addition to chow and water become leptin resistant therefore we investigated the effect of sucrose solution consumption on leptin signaling. In Experiment 1 rats were resistant to 3rd ventricle injections of 1.5 μg leptin after 36 days of sucrose and western blot indicated that resistance was associated with increased basal levels of signal transducer and activator of transcription 3 phosphorylation (pSTAT3). In Experiment 2 rats were resistant to a peripheral injection of 2 mg leptin/kg after 26 days of sucrose. Immunohistochemistry indicated that increased basal pSTAT3 was limited to the medial and lateral arcuate nucleus of the hypothalamus. Increased availability of glucose and fructose can stimulate the hexosamine biosynthetic pathway (HBP) which *O*-GlcNAc-modifies proteins. This has the potential to change protein bioactivity. We tested whether this pathway could account for the leptin resistance. There was no increase in the expression of HBP enzymes in tissues from sucrose rats in Experiment 1, however, direct activation of the HBP with a 3 h intravenous infusion of 30 μmol/kg/min glucosamine significantly increased hypothalamic pSTAT3. Although sucrose consumption and activation of the HBP both increase hypothalamic pSTAT3 experiments described here did not provide evidence of a direct link between sucrose consumption, HBP activity and leptin resistance. Unexpectedly, we found that the HBP enzyme glutamine fructose-6-phosphate amidotransferase (GFAT) in liver and *O*-GlcNAcase in hypothalamus were increased 30 min after leptin injection in leptin responsive animals, implying a complex interaction between activity of the HBP and leptin responsiveness.

1. Introduction

The importance of leptin, an adipose-derived cytokine, in the regulation of energy balance is well established. Animals and humans that have a deficit in leptin signaling or leptin production are hyperphagic, diabetic, infertile and obese [1–4]. In experimental conditions, peripheral or central administration of leptin inhibits food intake and weight gain of lean, chow-fed, wild type rats and mice [5–7]. By contrast, experimental animals that become obese and hyperleptinemic due either to aging [8] or consumption of a high-fat diet [9] are unresponsive to the effect of leptin administration on food intake. This lack of response to leptin is referred to as "leptin resistance" and has been attributed to a failure of leptin to cross the blood brain barrier [10], a decrease in the number of central leptin receptors [11,12] and increased expression of inhibitors of leptin receptor signaling [13]. Therefore, although the individual has high circulating concentrations of leptin, central receptors involved in the control of food intake are not fully activated.

There are multiple reports that feeding rats or mice a composite high-fat diet induces leptin resistance [9,14]. Some investigators have reported that rats offered a palatable high-fat diet become leptin resistant within only a few days [15,16] other have found that it can take months for resistance to develop [9,17]. We found that rats offered a

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choice diet in which they had free access to chow, 30% sucrose solution and lard increased their caloric intake and became resistant to both peripheral and central leptin administration within 18 days [18]. Subsequently we determined that access to 30% sucrose solution, chow and water is sufficient for the induction of leptin resistance [19]. In the experiments described here we have examined the impact of sucrose consumption on the hexosamine biosynthetic pathway (HBP) which has the potential to influence leptin production [20] and leptin responsiveness [21].

When glucose availability is increased activity of the "nutrient sensing" HBP is stimulated [22]. The end product of the pathway, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), is used for *O*-linked N-acetylglucosamine modification (*O*- GlcNAcylation) of threonine and serine residues in hundreds of bioactive proteins and transcription factors [23] (see Fig. 1). There is a significant literature showing a relation between increased activity of the HBP and development of insulin resistance [24–26] and it has been shown that this resistance is associated with *O*- GlcNAcylation of the insulin receptor and some of the proteins involved in the insulin signaling cascade [27,28]. In adipocytes increased activity of the HBP not only leads to insulin resistance [29], but also increases leptin expression and release [20]. Consistent with these observations, mice that over express glutamine:fructose-6-phosphate amidotransferase (GFAT), the enzyme that regulates entry of glucose into the HBP, are hyperleptinemic, but maintain a normal body fat mass [30], which implies that they are leptin resistant. We have previously reported a preliminary study that found that rats infused with glucosamine, which enters the HBP independent of GFAT, were resistant to the inhibitory effects of peripheral injection of leptin on food intake and weight gain [21]. The studies described here examine the changes in leptin signaling associated with sucrose-induced leptin resistance and further investigate the potential involvement of the HBP in this resistance.

2. Methods

Male Sprague Dawley rats weighing 275–300 g at the start of an experiment (Harlan Sprague Dawley, Indianapolis, IN) were used for these studies. The animals were housed in temperature controlled rooms at 70 °F with lights on for 12 h a day from 7.00 a.m. All animal procedures were approved by the Institutional Animal Care and Use Committee of Georgia Regents University.

Experiment 1. *The response to central leptin injections in sucrose rats*

The objective of this study was to examine the effect of leptin administration on activation of leptin signaling proteins in the hypothalamus and hindbrain of sucrose-consuming rats that had become leptin resistant and to determine whether there were changes in activity of the HBP in rats that consumed 30% sucrose solution. Thirty six rats were individually housed in hanging wire mesh cages. They were divided into two weight matched groups, one had free access to chow (Harlan Teklad Rodent Diet 8604) and water the other had access to chow, water and 30% sucrose solution (Kroger Sugar, Hood Packing Corporation, Hamlet, NC). The rats were weighed daily and chow and sucrose intake were recorded from Days 29 to 35. On Day 15 all of the rats were fitted with a 3rd ventricle guide cannula as described previously [31] and on Day 23 cannula placement was confirmed, as described previously [32]. On Days 26 and 36 central leptin responsiveness was tested. Food and sucrose were removed from the cages at 7.00 a.m. At 5.00 p.m. each rat received a 2 μl 3rd ventricle injection of either saline or 1.5 μg leptin (R&D Systems rat recombinant leptin, Minneapolis, MN). Food and sucrose were returned to the cages at 6.00 p.m. and energy intakes and body weights were recorded 14, 24, 38, 48 and 62 h after injection. Seven days later (Day 44) food and sucrose were removed from the cages at 7.00 a.m. and starting at 11.00 a.m. each rat received an i.p. injection of either saline or 1.0 mg leptin/kg body weight. Exactly 30 min later the rat was euthanized and trunk blood was collected for measurement of serum leptin (Rat leptin RIA EMD Millipore Corporation, Billericia, MA), glucose (Glucose assay kit GAGO20; Sigma-Aldrich, St. Louis, MO) glycerol (Free glycerol reagent F6428; Sigma-Aldrich), and triglycerides (TG: L-Type TG H kit; Wako Chemicals). Inguinal, epididymal, retroperitoneal and mesenteric fat depots and the liver were dissected and weighed. The inguinal fat and the liver were snap frozen for subsequent Western blot analysis of GFAT, O-linked N-acetylglucosamine transferase (OGT) , which catalyzes the addition of a single N-acetyl-D-glucosamine to serine or threonine residues of intracellular proteins, *O*-GlcNAcase which catalyzes the reversal of protein *O*-GlcNAcylation and *N*-acetyl-D-glucosamine (GlcNAc), as a measure of protein *O*-linked glycosylation. All western blot analyses followed the same general procedure as has been described previously [19]. The antibodies are listed below. One lobe of the liver was used to measure liver lipid content by chloroform methanol extraction. Tissue blocks of hypothalamic and hindbrain tissue were dissected and snap frozen, as described previously [33,34], and used for western blot analysis of pSTAT3(Tyr705), pSTAT3(Ser747), STAT3 suppressor of cytokine signaling 3 (SOCS3), GFAT, OGT, *O*-GlcNAcase and GlcNAc.

Experiment 2. *Hypothalamic pSTAT3 in rats given access to 30% sucrose solution*

We previously reported that rats given access to a 30% sucrose solution were resistant to the effects of peripheral injection of leptin on food intake and weight gain after three weeks on diet [19] and Experiment 1 showed an increase in basal pSTAT3(Tyr705) in sucrose rats. The objective of this experiment was to confirm those observations, to test the insulin responsiveness of the animals in glucose tolerance (GTT) and insulin tolerance (ITT) tests and at the end of the study to examine hypothalamic pSTAT3(Tyr705) in non-stimulated conditions. Fourteen rats were housed in individual wire-mesh cages with free access to water and chow. They were divided into two weight-matched groups and one group was offered free access to 30% sucrose solution in addition to chow and water. Body weight, chow intake and sucrose consumption were recorded daily except on Days 17 and 18 of the experiment. A GTT was performed on Day 12 of the experiment. Food and water were removed from the cages at 7.00 a.m. Starting at

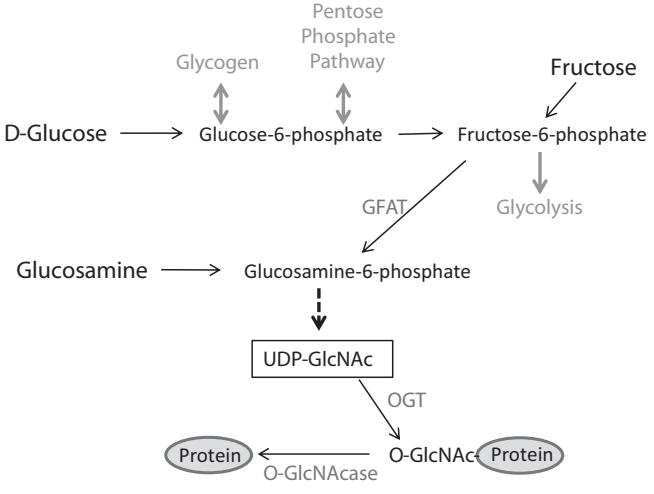

Fig. 1. A simplified schematic of the hexosamine biosynthetic pathway (HBP) identifying the point of entry of glucose, fructose and glucosamine into the pathway. GFAT = glutamine:fructose-6-phospahte aminotransferase, the enzyme which controls flux of glucose into the HBP. OGT = *O*-linked N-acetylglucosamine transferase, the enzyme which transfers a single N-acetylglucosamine by *O*-glycosidic (*O*-GlcNAc) linkage to serine or threonine residues of proteins. *O*-GlcNAcase = the enzyme that reverses *O*-GlcNAc protein modification.