Physiology & Behavior 139 (2015) 202–209

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Effects of sugar solutions on hypothalamic appetite regulation ☆

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HIGHLIGHTS

- Different sugars promote differences in hypothalamic appetite regulating proteins.
- Initial overnight access to a glucose solution upregulated hypothalamic CCK.
- Access to an equicaloric fructose solution downregulated hypothalamic CCK.
- High fructose corn syrup and sucrose had no effect on hypothalamic CCK message.
- Not all sugars are equally effective in affecting the controls of intake.

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 4 August 2014
Accepted 7 November 2014
Available online 14 November 2014

Keywords:
Food intake
Fructose
High fructose corn syrup
Glucose
Sucrose
Obesity

ABSTRACT

Several hypotheses for the causes of the obesity epidemic in the US have been proposed. One such hypothesis is that dietary intake patterns have significantly shifted to include unprecedented amounts of refined sugar. We set out to determine if different sugars might promote changes in the hypothalamic mechanisms controlling food intake by measuring several hypothalamic peptides subsequent to overnight access to dilute glucose, sucrose, high fructose corn syrup, or fructose solutions. Rats were given access to food, water and a sugar solution for 24 h, after which blood and tissues were collected. Fructose access (as opposed to other sugars that were tested) resulted in a doubling of circulating triglycerides. Glucose consumption resulted in upregulation of 7 satiety-related hypothalamic peptides whereas changes in gene expression were mixed for remaining sugars. Also, following multiple verification assays, 6 satiety related peptides were verified as being affected by sugar intake. These data provide evidence that not all sugars are equally effective in affecting the control of intake.

1. Introduction

Despite years of research, and billions of dollars spent every year in prevention and treatment, obesity has become and remains the nation's most preventable health problem [1,2]. The current obesity epidemic is not attributable to any one factor, and is not restricted by race, nationality, age or gender [3]. Despite its prevalence, there is no known cure for this disease. Several hypotheses about how obesity develops have been proposed that help guide research efforts. One such hypothesis is that dietary patterns of Americans have significantly shifted over the past fifty years by including unprecedented amounts of refined sugar [4]. Others have added that not all sugars are the same, and that fructose in particular accounts for much of the increase in sugar intake in the American diet [5,6]. From 1970 to 1990, consumption of high fructose corn syrup (HFCS) increased more than 1000% and currently accounts for 40% of all added caloric sweeteners [7,8]. Evidence that fructose is capable of promoting excessive weight gain has been reported in animal models and in humans. Rats maintained on a diet rich in HFCS for 6 or 7 months show abnormal weight gain, increased circulating TG and augmented fat deposition [9].

The hypothesis that fructose consumption is behind the obesity epidemic is not without its critics. Several groups have reported that when tested side-by-side, fructose is no more or less effective in promoting excess weight gain [10,11]. Moreover, the rise in circulating TG following fructose consumption can be explained by the metabolism of fructose. Fructose is metabolized differently than glucose, the more common monosaccharide. When glucose enters the cell there are multiple control points that regulate the conversion of glucose to fat (triglycerides). However these control points do not exist in the metabolism of fructose and thus fructose is readily metabolized into triglycerides [23,24].

In the laboratory, sugar-induced obesity can be studied by giving rats access to any of a wide variety of sugar solutions. Rats will consume approximately 60% of their total daily caloric intake from sugar solutions, despite differences in concentration and sweetness [12–14]. Long term access to sucrose, glucose, and fructose promote increased

☆ Funded in part by a grant from the Maryland Agricultural Experiment Station.
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http://dx.doi.org/10.1016/j.physbeh.2014.11.025
0031-9384/

weight gain and a shift in body composition favoring increased fat deposition [9]. Despite the extensive behavioral examinations of the rat's avidity for sugar solutions, relatively little work has been focused on the impact of sugar intake on the hepatic and central mechanisms controlling intake. Some exceptions to this characterization are from Erlanson-Albertsson and colleagues, who have demonstrated that fructose can upregulate fatty acid amide hydrolase, an enzyme involved in the degradation of hypothalamic endocannabinoids, as well as other enzymes involved in the synthesis of endocannabinoids [15]. Hoebel and colleagues have reported on the pronounced effects of sugar solutions on the release of dopamine in the nucleus accumbens (NAc), the brain's so-called "reward center" [16,17]. In addition to dopamine release, this group has demonstrated that sugar consumption can alter receptor gene expression in reward areas of the brain. We have more recently added to this literature by reporting that fructose consumption can promote increased oxoreductase activity of 11 beta hydroxysteroid dehydrogenase −1, an enzyme that regulates intracellular glucocorticoids in adipose tissue [18,19]. There are many such individual observations of the effects of one sugar or another on different areas of the brain. However, to the best of our knowledge, there has been no systematic screening of the effects of sugars on any one brain area.

The purpose of the present experiment was to directly compare the efficacy of fructose with high fructose corn syrup (HFCS-55), sucrose and glucose in altering the regulation of several of hypothalamic mechanisms that are known to control food intake. To do this, we used two sets of assays to establish and then confirm our findings. First, a commercially available PCR array system was used to evaluate 86 different obesity-related genes, using pooled cDNA templates. Once genes with significant changes were identified using the array, we verified those findings by using traditional RT-PCR analyses to probe the pooled hypothalamic cDNA templates used in the arrays. One critical difference between these procedures is that unlike the proprietary primers used in the arrays, these verification analyses used primers that were designed in-house.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley (CD strain) rats (Charles River Laboratories, Wilmington, MA) with a mean weight of approximately 300 g were used. Upon arrival, all animals were individually housed and maintained on a 12 h light/dark cycle with a room temperature of 22 °C ± 1 °C. During the 1 wk acclimation period the rats were given free access to the control diet and water. The animals were weighed and 24 h food intake and sugar solution intake (when appropriate) were measured daily at 0900 h throughout the experiment.

All rats were killed individually by slow replacement of air in a specialized chamber with pure CO_2 followed by rapid decapitation and exsanguination. This method has been approved for use by the Panel on Euthanasia of the American Veterinary Medical Association as well as the UM IACUC. All procedures described herein are in compliance with and have been approved by the University of Maryland's IACUC.

2.2. Animal diets

The control diet used in these experiments is based upon one previously used in an experiment that manipulated quality and quantity of fat in diet-induced obesity studies with rats [20]. The diet is a nutritionally complete low fat diet [Rodent diet 7012] prepared by Harlan Teklad (Bethlehem, PA) and provides 3.41 metabolizable kcal/g of diet. Per gram of chow, 2.14 kcal was derived from carbohydrate, 0.79 kcal was derived from protein, and 0.51 kcal was derived from fat. All animals were given free access to water throughout the experiment.

2.3. Study design

Rats (n = 44) were assigned to one of five weight-matched groups after an initial 1 wk acclimation period during which they had ad lib access to food and water. The rats assigned to the first group had ad libitum access to the control diet only, and served as the control group (n = 12). Rats assigned to the second group (n = 8) had ad libitum access to the control diet and free access to a 16% weight/volume (w/v) fructose (Tate & Lyle, Decatur, IL) solution. Rats assigned to the third group (n = 8) had ad libitum access to the control diet plus free access to a 16% (w/v) glucose (Sigma Aldrich, St Louis, MO) solution. Rats assigned to the fourth group (n = 8) had ad libitum access to the control diet and free access to 16% (w/v) HFCS-55 solution (Tate & Lyle, Decatur, IL), Finally, rats assigned to the fifth group (n = 8) had ad libitum access to the control diet and free access to a 16% w/v sucrose solution (Domino Foods, Baltimore, MD). All sugar solutions were prepared with tap water 24 h in advance and stored at 4 °C.

The rats were maintained on their respective diets for 24 h and then sacrificed. Truncal blood was collected, centrifuged, and serum separated and frozen. Brains were quickly dissected and flash frozen at −80 °C for subsequent use. The hypothalamus of each rat was sampled by sectioning each frozen brain using an IEC Minot Custom Microtome (Damon/IEC Division) and collecting 8 or 9, ~90 μm-thick consecutive tissue slices from the hypothalamic region starting at the interaural line +6.44 mm. Fig. 1 shows the hypothalamic region of interest.

2.4. Serum measures

Serum insulin concentrations were measured by ELISA (LINCOplex; LINCO Research). Serum glucose concentrations were measured enzymatically (Smith-Kline Beecham Laboratories). All reactions were run in duplicate.

A Dimension Clinical Chemistry System Flex reagent cartridge (Siemens Healthcare Diagnostics, Newark, DE) was used with a Dade Behring Dimension Xpand automated system to quantify plasma triglyceride levels. The assay is based on an enzymatic procedure using a combination of lipoprotein lipase, glycerol kinase, glycerol-3-phosphate-oxidase, and peroxidase. Changes in absorbance (510, 700 nm) resulting from the formation of quinoneimine from hydrogen peroxide reflect the total amount of glycerol and its precursors. All reactions were run in duplicate.

2.5. RNA extraction

Total RNA was extracted from each hypothalamic sample according to RNeasy Mini and RNeasy Lipid (QIAGEN) kit directions. The samples were purified with DNA-*free* (Ambion) and measured spectrophotometrically (Nanodrop) to determine concentration and check for quality.

2.6. cDNA synthesis

cDNA was made using the SABiosciences RT^2 First Strand Kit in accordance with instructions provided by SABioscience. Thirty-four uL of each sample was used to create the master stocks of cDNA from each experimental group, pooled by diet. These were used for both the arrays and pooled verification assays.

2.7. Overview of our strategy for the measurement of gene expression

Changes in gene expression were measured in two ways: RT^2 PCR arrays and pooled verification assays. First, hypothalamic cDNA from each group was pooled by group and served as the genetic material for the array. After completing the array, a list of several genes that were either up or down-regulated was created. These genes were used to follow-up using verification assays. Primers were designed for