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Research Report

Genes required for fructose metabolism are expressed in Purkinje cells in the cerebellum

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Abstract

Since 1967, fructose has become the primary commercial sweetener in the food industry. Large amounts of fructose can be toxic and have been correlated with atherosclerosis, malabsorption, hyperuricemia, lactic acidosis, and cataracts. To understand the deleterious and critical role(s) fructose plays in normal metabolism, it is essential to know how and where fructose is metabolized. The fructose transporter, GLUT5, and the specialized enzymes ketohexokinase, aldolase, and triokinase comprise the well-defined fructose-specific metabolic pathway found in liver, kidney, and small intestine. It is estimated that 50–70% of ingested fructose is metabolized in these tissues; where and how the remaining 30–50% is metabolized is not well defined. Prediction of tissues capable of metabolizing fructose via this pathway was done using expressed sequence tags (ESTs) in Unigene and a gene-specific virtual northern blot (VNB) algorithm. Unigene and VNB combined correctly predicted the expression of the genes required for fructose metabolism in liver, kidney, and small intestine. Both methods indicated brain, breast, lymphocytes, muscle, placenta, and stomach additionally express this set of genes. Expression of the genes for GLUT5 (*glut5*) and ketohexokinase (*khk*) in neurons was validated by immunohistochemistry and RNA in situ hybridization, respectively. Using stringent controls, clear expression of *glut5* and *khk* was localized to Purkinje cells in the cerebellum. Cerebellum was used to oxidize fructose to carbon dioxide. Together, these data suggest that these neurons in the brain are able to utilize fructose as a carbon source.

Theme: Other systems of the CNS
Topic: Brain metabolism

Keywords: Energy metabolism; Fructose; Bioinformatic; Transcriptional profile; Immunohistochemistry; RNA in situ hybridization

1. Introduction

Since the late 1960s, increasingly large amounts of fructose have found their way into the human diet [4], yet, a complete picture of how and where fructose is metabolized remains unknown [43]. Many problems may be associated with the ingestion of large amounts of fructose including hyperuricemia, lactic acidosis, cataracts, and obesity. Characterizing fructose metabolism is important considering the recent epidemic of obesity in Western cultures, which affects life expectancy and is associated with type II diabetes, hypertension, hyperlipidemia, and heart disease [15,43].

The major tissues for fructose metabolism are the liver and kidney, which extract 50–70% of fructose available from the portal blood [43]. Residual fructose is reportedly metabolized by tissues such as small intestine, adipose, and muscle, although the extent of fructose metabolism in these or other tissues is unknown [14]. Evidence suggests that other tissues may lack the specific enzymes used in the liver and kidney.

There are two commonly accepted pathways for fructose utilization (Fig. 1). The fructose 1-phosphate (Fru-1-P) pathway is utilized by the liver and kidney [24]. Other

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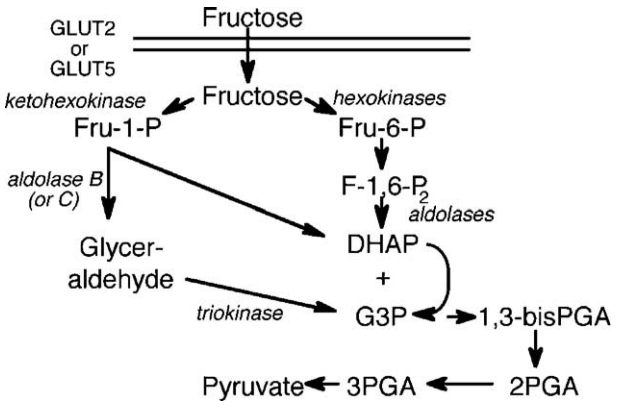

Fig. 1. Fru-1-P and Fru-6-P pathways for fructose catabolism. Pathways from fructose to pyruvate are depicted by arrows. Plasma membrane is indicated by double line. Transporters are all capital, and critical enzymes are lowercase italics. Fructose 1,6-bisphosphate (Fru-1,6-P_2), 1,3 bisphosphoglycerate (1,3-bisPGA), 2-phosphoglycerate (2PGA), and 3-phosphoglycerate (3PGA).

tissues do not express aldolase B or ketohexokinase and are thought to metabolize fructose via fructose 6-phosphate (Fru-6-P) by the action of a hexokinase, as proposed for adipose [14]. The issue whether hexokinase has any appreciable activity toward fructose in vivo, in the presence of glucose, remains controversial [40].

The Fru-1-P pathway for fructose metabolism starts with fructose absorption from the blood by the liver via GLUT2 (SLC2A2) and by the kidney via GLUT2 and GLUT5 (SLC2A5) [27]. Of these two transporters, GLUT5 is specific for fructose [31]. Any cells expressing GLUT5 should utilize fructose for either energy or secondary products such as sorbitol or fructose-3-phosphate [39]. Once in the cell, fructose is phosphorylated to Fru-1-P by ketohexokinase. The Fru-1-P is a substrate for aldolase, and the aldolase B isozyme has the lowest K_m for this substrate followed by aldolase C, then aldolase A [37]. The products of this reaction are dihydroxyacetone phosphate (DHAP), which enters the triosephosphate pool, and glyceraldehyde [24]. Glyceraldehyde enters glycolysis mainly at glyceraldehyde-3-phosphate (G3P) [25] via triokinase, which is also known as dihydroxyacetone kinase (DAK) in microorganisms [32], however, other pathways have been proposed and, unlike *khk* and *glut2/5*, it is not clear that this enzyme is absolutely required for fructose metabolism [43]. Clearly, those cells that express the genes necessary for this metabolism (*glut5*, *khk*, *aldo*, and possibly *dak*) would be capable of using fructose as a carbon source. While aldolases are well characterized and ubiquitous enzymes, GLUT5 and ketohexokinase are very specific enzymes for the Fru-1-P pathway with highly regulated gene expression. Concentrating on these pathway-specific enzymes, one can use transcriptional profiling methods to identify cells or tissues that coordinately express the Fru-1-P-pathway genes.

Transcriptional profiling has been used to detect gene expression patterns and to find novel genes [17]. One simple method for transcriptional profiling is to use the EST database (dbEST) [1]. Several sources for such information (Unigene [7], CGAP [34], TIGR [41]) are available and use refined BLAST-based algorithms for assembling ESTs into contigs (clusters). However, if the EST cluster is incorrect for any gene, such as closely related paralogs, the expression data can be misleading [17]. Nevertheless, recently tools have been developed to accurately mine dbEST for quantitative expression profiling [17].

In this study, the transcriptional profile for genes involved in the Fru-1-P pathway was determined by mining dbEST using Unigene and by virtual Northern blots (VNB). Besides the liver, kidney, and small intestine, expression of *glut5*, *khk*, *aldoB* and/or *aldoC*, and *dak* was observed in the brain, breast, and lymphocytes. Experimental validation of this pathway-specific bioinformatic approach was performed by immunohistochemistry and RNA in situ hybridization (RISH) in the brain. Significant expression of *glut5* and *khk* in Purkinje cells in the cerebellum was shown, and oxidation of fructose by the cerebellum suggests that these cells could metabolize fructose. This investigation introduces an approach for discovery of tissues and/or cell types engaged in fructose metabolism and will provide future targets for understanding any adverse effects of high fructose intake.

2. Materials and methods

2.1. Materials

Ampli-*Taq* DNA polymerase was purchased from PE-Express Cetus (Norwalk, CT). T7 RNA polymerase and DIG nucleic acid detection kit were from Boehringer Mannheim Biochemical (Indianapolis, IN). Oligodeoxyribonucleotides for PCR were from Midland Certified (http://www.oligos.com). Aquamount was purchased from Lerner Laboratories (Pittsburgh, Pennsylvania). Antibody to GLUT5 was a polyclonal goat anti-mouse GLUT5 (CAT# sc-14844) from Santa Cruz Biotechnology. All other chemicals were purchased from Sigma or Fisher Chemical (Fair Lawn, NJ).

2.2. Expression profiling using dbEST

The Unigene build number 186 at NCBI (http://www.ncbi.nlm.nih.gov/UniGene/) was searched using gene names as a keyword(s). The results were tabulated and sorted by summing ESTs from similar tissues. ESTs from cell lines and tumor or disease-derived tissues were not included in the analysis. The use of VNB followed the protocol described previously [17]. Briefly, gene-specific ESTs in dbEST for the Fru-1-P pathway were identified via BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) using 30 base segments of cDNA sequence (probes) derived from each of the genes. Unique probes were selected from regions along the entire full-length sequence. EST library informa-