Original Article

MOLECULAR METABOLISM

A gut–brain axis regulating glucose metabolism mediated by bile acids and competitive fibroblast growth factor actions at the hypothalamus

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ABSTRACT

Objective: Bile acids have been implicated as important regulators of glucose metabolism via activation of FXR and GPBAR1. We have previously shown that FGF19 can modulate glucose handling by suppressing the activity of hypothalamic AGRP/NPY neurons. As bile acids stimulate the release of FGF19/FGF15 into the circulation, we pursued the potential of bile acids to improve glucose tolerance via a gut–brain axis involving FXR and FGF15/FGF19 within enterocytes and FGF receptors on hypothalamic AGRP/NPY neurons.

Methods: A 5-day gavage of taurocholic acid, mirroring our previous protocol of a 5-day FGF19 treatment, was performed. Oral glucose tolerance tests in mice with genetic manipulations of FGF signaling and melanocortin signaling were used to define a gut–brain axis responsive to bile acids.

Results: The taurocholic acid gavage led to increased serum concentrations of taurocholic acid as well as increases of FGF15 mRNA in the ileum and improved oral glucose tolerance in obese (*ob/ob*) mice. In contrast, lithocholic acid, an FXR antagonist but a potent agonist for GPBAR1, did not improve glucose tolerance. The positive response to taurocholic acid is dependent upon an intact melanocortinergic system as obese MC4R-null mice or *ob/ob* mice without AGRP did not show improvements in glucose tolerance after taurocholate gavage. We also tested the FGF receptor isoform necessary for the bile acid response, using AGRP:*Fgfr1*−/− and AGRP:*Fgfr2*−/− mice. While the absence of FGFR1 in AGRP/NPY neurons did not alter glucose tolerance after taurocholate gavage, manipulations of *Fgfr2* caused bidirectional changes depending upon the experimental model. We hypothesized the existence of an endogenous hypothalamic FGF, most likely FGF17, that acted as a chronic activator of AGRP/NPY neurons. We developed two short peptides based on FGF8 and FGF17 that should antagonize FGF17 action. Both of these peptides improved glucose homeostasis after a 4-day course of central and peripheral injections. Significantly, daily average blood glucose from continuous glucose monitoring was reduced in all tested animals but glucose concentrations remained in the euglycemia range.

Conclusions: We have defined a gut–brain axis that regulates glucose metabolism mediated by antagonistic fibroblast growth factors. From the intestine, bile acids stimulate FGF15 secretion, leading to activation of the FGF receptors in hypothalamic AGRP/NPY neurons. FGF receptor intracellular signaling subsequently silences AGRP/NPY neurons, leading to improvements of glucose tolerance that are likely mediated by the autonomic nervous system. Finally, short peptides that antagonize homodimeric FGF receptor signaling within the hypothalamus have beneficial effects on glucose homeostasis without inducing hypoglycemia. These peptides could provide a new mode of regulating glucose metabolism.

Keywords Bile acids; FGF15; Hypothalamus; FGF receptors; AGRP; Melanocortins

1. INTRODUCTION

Bile acids modulate glucose homeostasis by direct actions on two receptors, GPBAR1 (G-protein coupled bile acid receptor 1; TGR5) [13] and FXR (farnesoid X receptor) [18,24,36]. GPBAR1's ability to stimulate incretin secretion, such as GLP1 and GLP2, from enteroendocrine cells is readily understood from its role as a G-protein coupled receptor [12]. While FXR has been thoroughly studied as a nuclear hormone receptor, FXR's sites and modes of action relevant to glucose homeostasis remain poorly understood [27]. One well known role for FXR is as a bile acid sensor [15] within the enterocyte that increases the production of FGF15 (FGF19 is the human ortholog). FXR acts as a direct transcriptional regulator of FGF15 and FGF19 due to binding sites within the promoter of the human *FGF19* gene [20]. FGFR4,

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among the four known FGF receptors, is the major hepatic isoform and is activated by circulating FGF15/19 to inhibit hepatic synthesis of bile acids from cholesterol [40]. However, FGF19 can significantly improve glucose handling even in mice that do not express FGFR4, indicating that other FGF receptors found in extrahepatic tissues are critical.

Antibodies to FGFR1 [32], which have mixed agonist/antagonist activity, improve glucose tolerance while accumulating within the median eminence, pointing to a central site of action. We have shown that FGF19 suppresses the activity of hypothalamic neurons which express Agouti gene related peptide (AGRP) and Neuropeptide Y (NPY) neurons [19], suggesting that modulation of central melanocortinergic tone is the critical mechanism for the ability of FGF19 to improve glucose homeostasis.

Previous genetic studies have revealed pleiotropic actions for FGF receptors, principally during development [9]. Activating mutations of *FGFR1*, *FGFR2*, and *FGFR3* cause syndromes with craniosynostosis as a major feature [34]. Complete absence of FGFR1 or FGFR2 causes developmental abnormalities of the central nervous system and is typically incompatible with life. As an interesting side note, the FGF8/FGFR1 ligand/receptor pair [26] is critical for the migration and maturation of gonadotrophin releasing hormone cells from the olfactory placode with resultant hypothalamic hypogonadism associated with mutations in *FGF8* or *FGFR1*.

In this report, we conduct studies with oral bile acid supplementation to provide evidence that bile acid signaling via FXR affects ileal FGF15/19 production, causing activation of FGFR1 on melanocortinergic neurons to significantly improve glucose tolerance. We propose that there is a gut—brain axis that can modulate glucose metabolism, an axis that incorporates 1) small molecules (bile acids) that are synthesized by the liver and undergo biotransformation by gut bacteria and the enterohepatic circulation and 2) a receptor, FGFR1, on hypothalamic neurons which can activate intracellular signaling pathways overlapping those triggered by leptin and insulin. Further testing of our hypothesis suggested that some FGF ligands might worsen glucose handling. Postulating that antagonism between FGF ligands (FGF15/19/21) that bind to beta Klotho-FGF receptor heterodimers and FGF ligands (FGF8/17/18) that bind to FGF receptor homodimers, we tested the ability of small peptides (predicted to block FGF receptor signaling) based on FGF8 and FGF17 to improve glucose handling. These short peptides improved glucose handling as measured with glucose tolerance tests as well as lowering daily average blood glucose after central and peripheral administration. In conclusion, we have identified a gut—brain axis wherein bile acid and FGF15/19 can improve glucose handling as well as identifying short FGF-derived peptides that improve glucose homeostasis.

2. MATERIALS AND METHODS

2.1. Animals and animal handling — genotyping, gavage, GTT and ITT

Animals were housed and bred in the Einstein barrier facility with 12 h light/12 h dark (6 am EST lights on) and provided with water and chow (Purina 5058) *adlib* in polycarbonate cages with corn cob bedding. Heterozygous *ob*/+ mating pairs generated *ob/ob* animals with *Npy-hrGFP* [35] segregating in the colony. A separate colony of *ob*/+ *Agrp*−/− mice were used to generate *ob/ob Agrp*−/− mice [11]. Heterozygous *Mc4r*+/− mice were used to generate *Mc4r*−/− animals. The *Mc4r* null allele was generated from CRE-mediated deletion of the *Mc4r-flox* allele [1]. *Fgfr1-flox/flox Agrp-CRE* mice were generated from female *Fgfr1-flox/flox* [25]and *Fgfr1-flox/flox Agrp-CRE* males. While it has been suggested that the *Agrp-IRES-CRE* knock-in allele causes fewer instances of extrahypothalamic deletion, the presence of both *Agrp* (Chr. 8, 53 cM) and *Fgfr1* (Chr. 8, 14 cM) on mouse Chromosome 8 made the production of the double mutant mice extremely difficult, necessitating the use of the *Agrp-CRE* BAC-based transgene. *Fgfr2-flox/flox Agrp-IRES-CRE* mice were generated from female *Fgfr2-flox/flox* [6] and male *Fgfr2-flox/flox Agrp-IRES-CRE* mice. Genotypes were determined with DNA isolated from ear clips.

For bile acid gavage, sodium taurocholate (Sigma—Aldrich, St. Louis, MO) dissolved in water was dosed at 3 mg/g of body weight [10] while lithocholic acid [29] (sigma-Aldrich, St. Louis, MO) suspended in peanut oil was dosed at 0.1 mg/g of body weight. These doses were based on published reports of known efficacy for activation of FXR and GPBAR1, respectively. Bile acid gavages were given daily in the morning for 5 days. On the 5th day, overnight fasted mice were challenged with glucose. Oral glucose tolerance tests were done as previously described [19] with glucose loads of 0.5 mg/g for genetically obese mice (*ob/ob*, *ob/ob Agrp*−/− and *Mc4r*−/−) and 2 mg/g for mice without genetic obesity mutations. Glucose was determined from tail vein blood samples using the glucose oxidase method (Control AST, US Diagnostics, New York, NY). Insulin was determined from sera obtained from fasting mice using a quantitative ELISA kit with mouse insulin standards (Crystal Chem, Downers Grove, IL).

Peptide injections (FGF8 and FGF17; Novoprotein, Summit, NJ) were performed icv via an indwelling third ventricular cannula. FGF8 was resuspended in aCSF at 1 mg/ml, and mice were infused with 1 μg of the peptide via syringe pump. FGF-derived peptides (F8b13, F17b13, F8b13ProPro; sequences in Figure 6) were custom synthesized by GenScript (Piscataway, NJ) at 90% purity. These peptides, initially prepared at 1 mg/ml, were diluted to 100 μg/ml in aCSF, and mice were infused with 100 ng of peptide via syringe pump. For ip injections, these peptides were injected ip at 10 μg/g (body weight). Continuous glucose monitor devices from Data Scientific International (DSI, St. Paul, Minnesota) were implanted into the ascending aorta (glucose detector) and the temperature monitor, activity monitor, and battery were implanted into the abdomen. Signals were collected continuously for >4 weeks or until the batteries were discharged. Calibration was performed with tail blood using a portable glucometer weekly. Data were analyzed in 24 h windows. Daily average blood glucose values were the mean of glucose values collected per minute over 24 h (1440 data points per mouse per day) although the device can be programmed for data collection at shorter time intervals. All procedures had been approved by the Einstein Institutional Animal Care and Use Committee.

2.2. Quantitative RT-PCR and hormone assays

RNA isolated from the terminal ileum was used for quantification of FGF15 mRNA by quantitative RT-PCR. All RNA samples underwent quality checks by agarose gel electrophoresis and quantitation by absorbance readings at 260 nm. Briefly, RNA was reverse transcribed to generate first strand cDNA with Mo-MuLV reverse transcriptase (Superscript III, Life Technologies, Carlsbad, CA) which was used as a template for quantitation by SYBR Green fluorescence during Taq polymerase based amplification on a Roche LightCycler 480II. All primer pairs (synthesized from Integrated DNA technologies, Coralville, IA) spanned at least on intron to prevent amplification of genomic DNA. Relative quantitation was based on the delta—delta Ct method using beta 2 microglobulin mRNA as a loading control. Untreated control means were set to 1.0 for comparison purposes and treated samples were expressed relative to those means.

