FGF21 Regulates Sweet and Alcohol Preference

Highlights

- FGF21 suppresses sweet and alcohol preference but not bitter taste
- The effects of FGF21 on taste preference require its action in the CNS
- FGF21 decreases dopamine levels in the nucleus accumbens
- FGF21 regulation of sweet preference also occurs in primates

In Brief

FGF21 has well-established beneficial metabolic effects. Talukdar et al. now extend this repertoire and reveal that FGF21 also suppresses sweet and alcohol preference in mice, and sweet preference in monkeys, by acting on the CNS. These effects are associated with decreased dopamine, a key neurotransmitter used in reward pathways.

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In Brief

FGF21 has well-established beneficial metabolic effects. Talukdar et al. now extend this repertoire and reveal that FGF21 also suppresses sweet and alcohol preference in mice, and sweet preference in monkeys, by acting on the CNS. These effects are associated with decreased dopamine, a key neurotransmitter used in reward pathways.

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INTRODUCTION

FGF21 acts through a cell-surface receptor composed of a conventional FGF receptor in complex with β-Klotho, a single-pass transmembrane protein (Owen et al., 2015). FGF21 crosses the blood-brain barrier (Hsuchou et al., 2007) and exerts many of its actions, including its effects on growth, female reproduction, and weight loss, by acting on its cognate receptor in the CNS (Bookout et al., 2013; Liang et al., 2014; Owen et al., 2013, 2014). Among its central actions, FGF21 induces corticotropin-releasing factor and suppresses arginine vasopressin expression in the hypothalamus (Bookout et al., 2013; Liang et al., 2014; Owen et al., 2013, 2014). In humans, SNPs in and around the FGF21 gene are associated with changes in macronutrient preference, including increases in carbohydrate consumption and decreases in fat and protein intake (Chu et al., 2013; Tanaka et al., 2013). These findings raise the possibility of additional effects of FGF21 on the brain. In this report, we examine the effect of FGF21 on sweet and alcohol preference in mice and monkeys.

RESULTS AND DISCUSSION

Since FGF21 is induced by carbohydrates in rodents and humans (Dushay et al., 2015; Sánchez et al., 2009), and SNPs in the FGF21 gene are associated with carbohydrate intake in humans (Chu et al., 2013; Tanaka et al., 2013), we investigated whether chronic FGF21 exposure affects sweet preference. Two-bottle preference assays with water and either 3% sucrose or 0.2% saccharin (Kishnani et al., 2007; Tordoff and Bachmanov, 2003) were performed using wild-type (WT) and Fgf21-transgenic (Tg) mice expressing supraphysiological concentrations of FGF21 (Inagaki et al., 2007). Saccharin was included to eliminate the potentially confounding effect of caloric content. As expected, WT mice showed a strong preference for drinking water sweetened with either sucrose or saccharin (Figures 1A and 1B, Table S1). Notably, the Tg(Fgf21) mice had significant decreases in both sucrose and saccharin preference (Figures 1A and 1B).
FGF21 decreases sweet preference in both contexts. In addition, two-bottle preference tests, FGF21 had no effect on preference for 1% sunflower oil (data not shown) or quinine (Figure 1D), indicating that FGF21 does not affect responses to fatty acids or bitter taste. FGF21 administration also had no effect on either tail-suspension or forced-swim tests, both standard measures of behavioral despair (Figures S1A and S1B). We conclude that FGF21 acts directly on the brain to regulate sweet preference without causing despair.

To determine whether FGF21 also affects sweet preference in primates, we analyzed saccharin preference in obese cynomolgus monkeys administered PF-05231023, a long-acting FGF21 analog consisting of two molecules of modified FGF21 linked by an antibody scaffold (Dong et al., 2015; Giragossian et al., 2015; Weng et al., 2015). We first tested this analog in mice. PF-05231023 administration decreased saccharin preference to a degree similar to native FGF21, with maximal efficacy observed 3–5 days after dosing (Figure 2A). For the monkey study, PF-05231023 or vehicle was administered on days 1, 4, and 7 of the 3-week experiment. Notably, PF-05231023 administration significantly decreased saccharin preference in the monkeys (Figure 2B). The effect on saccharin intake in the monkeys was striking even within 1 day of receiving a single dose of the FGF21 analog, and the effect was sustained for several days after receiving the last dose. Thus, FGF21 also affects sweet preference in primates.

The neurotransmitter dopamine has a central role in regulating reward behavior, including sucrose and saccharin preference (Fernstrom et al., 2012). To examine whether FGF21 affects dopamine signaling, including the mesolimbic pathway, we first measured β-Klotho expression in the ventral tegmental area (VTA), nucleus accumbens (NAC), medial prefrontal cortex (PFC), and caudate putamen (CP) of Klb−/− and Klb+/− mice, with the latter mice serving as a negative control. As expected (Bookout et al., 2013; Liang et al., 2014), Klb mRNA was detected in the suprachiasmatic nucleus/paraventricular (SCN/PVN) nucleus region of the hypothalamus in Klb+/− mice by quantitative PCR (qPCR) (Figure 3A). Klb mRNA was also detected in VTA and NAC in Klb+/− mice, albeit at relatively low levels, but not the PFC or CP (Figure 3A). As expected, Klb mRNA was not detected in any of the regions in the Klb−/− control mice (Figure 3A). Consistent with the qPCR data, Klb mRNA was detected by in situ hybridization in SCN and a small subset of cells in the VTA and NAC (Figure S2A). Expression of FGF receptor 1, which partners with β-Klotho to form the FGF21 receptor, was detected by qPCR in all of these brain regions (Figure S2B).

The FGF21 receptor expression data led us to examine whether FGF21 affects the levels of dopamine and its metabolites in NAC, which coordinates reward behaviors. Notably, FGF21 administration for 2 weeks significantly decreased dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) concentrations (Figure 3B). FGF21 administration did not decrease dopamine, DOPAC, HVA, or 3-MT concentrations in the CP (Figure S2C). FGF21 administration also caused changes in the expression of dopamine-related genes, including an increase in the dopamine transporter in the NAC and CP and a decrease in catechol-O-methyl transferase in the VTA and CP (Figures 3C and 3D). FGF21 had little or no effect on the mRNA levels of tyrosine hydroxylase or dopamine receptor 1 in these brain regions (Figures S2D and S2E), nor did it change the levels of total and...
phosphorylated tyrosine hydroxylase in VTA (Figure S2F). Taken together, these data suggest that FGF21 may affect sweet preference via effects on dopamine signaling. However, additional experiments will be required to confirm this possibility and to determine the mechanism whereby FGF21 reduces dopamine concentrations.

Since dopamine signaling impacts ethanol drinking behavior (Gonzales et al., 2004), we examined whether FGF21 also affects alcohol preference. Groups of WT and Tg(Fgf21) mice were exposed stepwise to increasing concentrations of ethanol in a two-bottle preference assay. Tg(Fgf21) mice had a decreased ethanol preference ratio at the 4%, 8%, 12%, and 16% ethanol concentrations (Figure 4A, Table S3). In an ethanol bioavailability test, there was no difference between WT and Tg(Fgf21) mice in plasma ethanol concentrations at 1 and 3 hr after ethanol administration (Figure 4B). Thus, FGF21 suppresses ethanol preference without affecting its bioavailability.

In summary, we show that FGF21 regulates sweet and alcohol preference in mice and sweet preference in monkeys. Since circulating levels of FGF21 increase in response to carbohydrate consumption in rodents and humans (Dushay et al., 2015; Sánchez et al., 2009) and alcohol consumption in rodents (Zhao et al., 2015), this may represent a feedforward regulatory pathway for limiting consumption. In mice, the effects on sweet and alcohol preference correlate with reductions in dopamine concentrations in the NAc, which coordinates reward behavior. These results suggest a mechanistic basis for the association between SNPs in and around the FGF21 gene with macronutrient preference in humans (Chu et al., 2013; Tanaka et al., 2013). Moreover, since FGF21 is currently in clinical trials for treating obesity and type 2 diabetes, these findings suggest that additional studies are warranted to assess the effects of FGF21 on sweet and alcohol preference and other reward behavior in humans.

**EXPERIMENTAL PROCEDURES**

**Mouse Experiments with FGF21**

All mouse experiments involving native FGF21 were approved by the Internal Animal Care and Use Committee of the University of Texas Southwestern Medical Center. WT and Tg(Fgf21) mice were on a C57BL/6J background. Klbfl/fl and KlbCamk2a mouse experiments were performed with littermates. Mice were housed on a standard 12 hr light/dark cycle and had free access to chow. Recombinant human FGF21 protein was provided by Novo Nordisk and administered by subcutaneous osmotic minipumps (Alzet) at a dose of 1 mg/kg/day. Mice were allowed to recover from minipump surgery for 1 week prior to preference tests. Mice were single-caged following minipump surgery, which was conducted under isoflurane anesthesia and 24 hr buprenorphine analgesia.

**Two-Bottle Preference Assays**

For the two-bottle sucrose and saccharin preference assays, mice were acclimated to cages with two bottles of just water for 4 days. Mice were then given access to bottles with water and water containing 3% sucrose or 0.2% saccharin (w/v). For the quinine preference assay, mice were given access to water and water containing 2 mg/dl quinine. In each case, consumption was measured daily for at least 3 days. For ethanol preference assays, mice were given access to two bottles, one containing water and the other containing ethanol (v/v) in water. The same mice were exposed to an ascending concentration of each ethanol concentration for 5 days. The position of the two bottles was changed every 2 days to exclude position effects. Water and ethanol-containing water intake were measured each day.

**Mouse Immobility Assays**

Tail-suspension and forced-swim tests were performed as described (Can et al., 2012a, 2012b) on mice administered either FGF21 or vehicle by osmotic minipump for 7–14 days. In both tests, the experimenter was blinded to the treatment group. For the forced-swim test, mice were placed in cylindrical tanks (20 cm in diameter) filled with water (25 ± 2°C). The cylinder was filled to a depth of 12 cm to prevent the mice from using their tails to support
themselves in the water. In both experiments, the cumulative time spent immobile was recorded over the course of a 6 min experiment.

**Ethanol Clearance Assays**

Mice were intraperitoneally (i.p.) injected with ethanol (4 g/kg) in saline, and tail vein blood was drawn at regular intervals. Plasma ethanol levels were measured using the EnzyChrom Ethanol Assay Kit (BioAssay Systems).

**Microdissection of Brain Regions**

Mouse brains were extracted from the skull and kept under dry ice vapor for all dissections. Coronal sections (1-mm thick) were cut using a brain-slicing matrix (Braintree Scientific). Medial prefrontal cortex, whole NAc (shell and core), the hypothalamic SCN/PVN region, CP (striatum), and VTA were identified by gross architectural landmarks (Paxinos and Franklin, 2004). Medial prefrontal cortex and the hypothalamic SCN/PVN region were dissected using a 14G tissue punch. NAc, CPA, and the VTA were dissected using a 16G tissue punch.

**Figure 3. FGF21 Affects Dopamine Signaling**

(A) **Klb** mRNA levels in the suprachiasmatic nucleus/paraventricular nucleus (SCN/PVN) region of the hypothalamus, ventral temporal area (VTA), nucleus accumbens (NAc), medial prefrontal cortex (PFC), and caudate putamen of **Klb** and **Klb** mice (n = 6/group). Ct values are shown in the bars. ND, not detected.

(B) Concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) in the NAc of mice administered either vehicle or FGF21 for 2 weeks by osmotic minipump (n = 12/group).

(C and D) mRNA levels of dopamine transporter (**Slc6a3**) or (D) catechol-O-methyl transferase (**Comt**) in VTA, NAc, and caudate putamen of mice administered either vehicle or FGF21 for 2 weeks by osmotic minipump (n = 7–8/group). Ct values are shown.

Values are means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus control group by Student’s t test. See also Figure S2.

Tissue was homogenized by passage through a 26.5G syringe in RNA-STAT60.

**Quantitative PCR Analysis**

Total RNA was isolated from tissue using RNA-STAT60 reagent, and RNA was reverse-transcribed into cDNA (Invitrogen). Gene expression was measured with an Applied Biosystems 7900HT Sequence Detection System using the **DD** assay and normalized to GAPDH.

**In Situ Hybridization Analysis**

Brains were dissected from male C57BL/6J mice, embedded in OCT compound (Sakura), and flash frozen in cooled isopentane. Coronal sections (14–16 μm) were cut using a cryostat (Leica) followed by fixation for 15 min at 4°C with 10% neutral buffered formalin. In situ hybridization was performed using the RNAscope 2.5 brown chromogenic assay pretreatment and detection kits (Advanced Cell Diagnostics). Probes for cyclophilin B (positive control), dapB (negative control), and Klb were purchased from Advanced Cell Diagnostics. Hybridized sections were counterstained with hematoxylin, dehydrated, cleared, and mounted with Ecomount (Biocare Medical). Images were taken using a Zeiss Axioscan Z1 at 40x magnification. The signal from the mRNA was highlighted using the color threshold function in ImageJ.

**Western Blot Analysis**

Western blot analysis was performed using antibodies for total (Cell Signaling, #2792) and Ser40-phosphorylated tyrosine hydroxylase (AbCam, #51206). Data were acquired and quantified using an ImageQuant LAS 4000 and Multi Gauge v3.1 software (Fujifilm).

**Dopamine Measurements**

Dopamine and its metabolites were measured by HPLC by the Vanderbilt Neurochemistry Core.

**Mouse and Monkey Experiments with PF-05231023**

All animal care and experimental procedures for studies involving PF-05231023 were conducted in compliance with the U.S. Animal Welfare Act.

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The monkeys underwent a training and consumption assay performed as described (Tordoff and Bachmanov, 2003). The two-bottle saccharin preference assay was conducted as follows: standard lab diet 5K91 (LabDiet) supplemented once a day with fruits and vegetables was provided to the animals. Mice were housed under a 12 hr light/dark cycle, and the ILAR Guide for the Care and Use of Laboratory Animals, 1996. The procedures used in these studies were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee (AUP #GTN-2013-00793).

Statistical Analyses
All data are presented as means ± SEM. Statistical analysis between the two groups was performed by unpaired two-tailed Student’s t test using Excel or GraphPad Prism (GraphPad Software, Inc.) or by using R software (Frawley et al., 2012; Pinheiro et al., 2013).

SUPPLEMENTAL INFORMATION
Supplemental Information includes two figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.12.008.

REFERENCES


AUTHOR CONTRIBUTIONS
S.T., B.M.O., P.S., G.H., and Y. Zhou designed, performed and analyzed experiments; Y. Zhang and W.T.S. performed and analyzed experiments; B.P., T.T., A.S., and B.B. performed experiments; H.T. analyzed experiments; C.P.M. designed and analyzed experiments; B.G., S.A.K., and D.J.M. designed, supervised, and analyzed experiments and wrote the paper. P.S. and G.H. contributed equally. All authors commented and approved the paper.

CONFLICTS OF INTEREST
S.T. and Y. Zhou completed the work at Pfizer and are now employees of Merck; B.P., T.T., A.S., B.B., and B.G. are employees of Pfizer; D.J.M. is a founder of Metacrine and a member of its scientific advisory board; the other authors have no conflicts of interest to declare.

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