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Monoclonal antibody antagonists of hypothalamic FGFR1 cause potent but reversible hypophagia and weight loss in rodents and monkeys

Haijun D. Sun,1 Maria Malabunga,1 James R. Tonra,1 Roberto DiRenzo,1 Francine E. Carrick,1 Huiyuan Zheng,2 Hans-Rudolf Berthoud,2 Owen P. McGuinness,3 Juqun Shen,1 Peter Bohlen,1 Rudolph L. Leibel,4 and Paul Kussie1

1ImClone Systems Incorporated, New York, New York; 2Neurobiology of Nutrition Laboratory, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana; 3Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville Tennessee; and 4Division of Molecular Genetics, Naomi Berrie Diabetes Center, New York, New York

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Monoclonal antibody antagonists of hypothalamic FGFR1 cause potent but reversible hypophagia and weight loss in rodents and monkeys. Am J Physiol Endocrinol Metab 292: E964–E976, 2007. First published November 28, 2006; doi:10.1152/ajpendo.00089.2006.—We generated three fully human monoclonal antibody antagonists against fibroblast growth factor receptor-1 (FGFR1) that potently block FGF signaling. We found that antibodies targeting the c-splice form of the receptor (FGFR1c) were anorexigenic when administered intraperitoneally three times weekly to mice, resulting in rapid, dose-dependent weight loss that plateaued (for doses >4 mg/kg) at 35–40% in 2 wk. Animals appeared healthy during treatment and regained their normal body weights and growth trajectories upon clearance of the antibodies from the bloodstream. Measurements of food consumption and energy expenditure indicated that the rapid weight loss was induced primarily by decreased energy intake and not by increased energy expenditure or cachexia and was accompanied by a greater reduction in fat than lean body mass. Hypophagia was not caused through malaise or illness, as indicated by absence of conditioned taste aversion, pica behavior, and decreased need-induced salt intake in rats. In support of a hypothalamic site of action, we found that, after intraperitoneal injections, anti-FGFR1c (IMC-A1), but not a control antibody, accumulated in the median eminence and adjacent mediobasal hypothalamus and that FGFR1c is expressed in the pituitary and the hypothalamus (17). In the pituitary, they are present in the neutral and anterior lobes; in the hypothalamus, they are present in the magnocellular neurons of paraventricular and supraoptic nuclei, as well as the median eminence. It has been hypothesized that hypothalamic FGF signaling may be involved in the regulation of hypothalamic energy homeostasis; hypothalamic; fibroblast growth factor receptor; food intake; obesity

THE FGF-FGFR AXIS (fibroblast growth factor-fibroblast growth factor receptor) plays a central role in embryonic development, osteogenesis, tissue maintenance, and repair (24, 42, 46). FGF signaling is complex: at least 22 ligands are known that signal through four distinct cell surface receptors (FGFR1, -2, -3, and -4). Some FGF-knockouts (such as FGFR4, -8, -9, -10, -18 and FGFR1) are embryonic lethal; others generate relatively mild phenotypes due to considerable redundancy in the signaling pathway(s) (10, 24, 42, 46). Loss-of-function mutations in FGFR1 have been implicated in instances of hypogonadotrophic hypogonadism (Kallmann syndrome), indicating a role for the receptor in human central nervous system (CNS)/hypothalamic development (45). FGF ligand-receptor binding induces receptor dimerization and autophosphorylation, leading to downstream activation of effector molecules such as mitogen-activated protein kinase (MAPK). In FGFR1, -2, and -3, alternative splicing of the exons encoding the third IgG-like domain produces either the b- or the c-splice form of the receptors. The various splice forms exhibit distinctive ligand-binding preferences. Therefore, spatial and temporal expression of the splice forms and their ligands may serve as an important control mechanism for FGF signaling (18, 24).

During brain development, FGF2, -3, -4, -8, and -17 and FGFR1, -2, and -3 are expressed in neural cells and adjacent inducing tissues and constitute part of an elaborate cell fate switch mechanism during the formation of the vertebrate CNS. In adult brain, FGF2 is an important neurotrophic factor that contributes to neuron survival and lesion repair. FGFR1 and -2 may also play a role in the regulation of synaptic plasticity (Refs. 10, 17, 36, and 48 and references therein). FGF2 increases the number of excitatory synapses between cultured embryonic hippocampal neurons (29) and enhances long-term potentiation of neuronal signaling (1). FGF2 and FGFR1 are expressed in the pituitary and the hypothalamus (17). In the pituitary, they are present in the neutral and anterior lobes; in the hypothalamus, they are present in the magnocellular neurons of paraventricular and supraoptic nuclei, as well as the median eminence. It has been hypothesized that hypothalamic FGF signaling may be involved in the regulation of hypothalamic functions, particularly water balance (17).

We generated functionally blocking anti-FGFR antibodies to determine their potential in cancer therapy. Unexpectedly, we found that peripherally administered antibodies targeting FGFR1c caused profound weight loss. Herein we describe the initial biological characterization of these antibodies. Suspecting that the weight loss effect was mediated through the CNS, we determined whether peripherally administered antibodies could be detected in the hypothalamus, a CNS center of energy homeostasis regulation (6, 34, 61), and whether antibodies injected intracerebrally had the same effects on energy homeostasis. Others have shown that intracerebroventricular (icv) injection of FGFI or -2 decreases food intake (Refs. 20, 22, 40, and 41 and references therein) and that polyclonal antibodies against either ligand or FGFR1 induce weight gain (28, 52).

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Our results confirm the involvement of FGF signaling in hypothalamic regulation of energy homeostasis but demonstrate an anorexigenic effect when the specific signaling of FGFR1c is inhibited.

MATERIALS AND METHODS

Materials

Human FGFR-Fc, sEGFR, PDGFR-Fc, and sFGF1-R recombinant proteins were purchased from R&D Systems (Minneapolis, MN). Recombinant human FG2, custom-synthesized primers, T4 DNA ligase, Platinum PCR SuperMix High Fidelity kit, pUC19 vector, pCDNA3 vector, Max Efficiency DH5α competent cells, and FreeStyle 293 system were purchased from Invitrogen (Carlsbad, CA). Recombinant mouse FGFR1c-AP (15H1c) and full-length human FGFR1b (13I2) vectors were kindly provided by Dr. David Ornitz (Washington University, St. Louis, MO). Human FGFR1c cDNA, T36274 mouse skin cells, and L6 myoblast cells were purchased from American Type Culture Collection (Manassas, VA). All restriction enzymes and DNA polymerases were purchased from New England Biolabs (Beverly, MA). Rabbit anti-phosphotyrosine and anti-FGFR1 antibodies were purchased from Calbiochem (San Diego, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Horseradish peroxidase (HRP)-conjugated anti-human antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). Nonspecific human IgG and monoclonal anti-human placental alkaline phosphatase-agarose beads were purchased from Sigma (St. Louis, MO). Humanized chimeric monoclonal antibody against epidermal growth factor receptor C225 (47) is produced by ImClone Systems (New York, NJ). OTC medium was purchased from VWR (Bridgeport, NJ). Protein A/G beads were purchased from GE Health Care Bio- science (Piscataway, NJ).

Expression of soluble recombinant murine FGFR1-alkaline phosphatase (AP) proteins. Murine FGFR1c-AP cDNA was subcloned from the parent vector (pCDNA3) into pUC19 using HindIII and BamHI. To construct a recombinant murine FGFR1b-AP pDNA vector, murine FGFR1b splicing variant sequence was cloned from T36274 mouse skin cell FGFR1b cDNA (3) using two PCRs with the following primers: forward 5′-gttagacggctgacctca-3′ (BsrGI site underlined) and reverse 5′-ggggttctgacggctgcc-3′; forward 5′-ggaagcattcgggaattaatag-3′, and reverse 5′-ggagctcctgactgtg-3′ (BglII site underlined). The PCR products were annealed and filled in to yield a 300-bp sequence. This sequence was amplified with the two primers containing the engineered restriction enzyme sites, and the BsrGI/BglII product was spliced into the murine FGFR1c-AP pUC19 vector to replace the FGFR1c variant sequence. The resulting plasmid was treated with HindIII/BamHI, and the FGFR1b-AP sequence was isolated and cloned into a pCDNA3 vector. The authenticity of the FGFR1b-AP construct was verified by sequencing.

The murine FGFR1c-AP and FGFR1b-AP vectors were used to express soluble recombinant FGFR1 in the FreeStyle 293 system. Proteins of interest were purified from the medium using affinity chromatography with monoclonal anti-human placental AP-agarose beads.

Generation of L6 cell lines expressing human FGFR1b or FGFR1c. Human FGFR1b or FGFR1c cDNAs were amplified from the parent vectors using primers forward 5′-gactgattgagcatcggctgcc-3′ (BamHI site underlined) and reverse 5′-gactgattgagcatcggctgcc-3′ (SacI site underlined). The BamHI/SacI product was cloned into the pBABE expression vector containing the puromycin selection gene (39). Retroviral expressions of resulting plasmids in L6 cells were performed. Cells were selected and cultured in DMEM medium containing 10% FBS and 2 μg/ml puromycin.

Generation of Anti-FGFR1 Antibodies

Anti-FGFR1 Fab clones were obtained by panning a naive human bacteriophage Fab library against recombinant human FGFR1b or FGFR1c, engineered into IgG1 antibodies, expressed, and purified according to the protocols described previously (8). Three anti-FGFR1 antibodies were obtained: IMC-4H, IMC-A1, and IMC-H7, which specifically target the b-, c-, and both splicing forms of FGFR1, respectively. The binding kinetics of these antibodies to recombinant FGFR1 (human and murine) and FGFR2, -3, and -4 (human only) were determined using a BiaCore 3000 biosensor (BiaCore, Piscataway, NJ).

FGF ligand receptor-binding ELISA. Each well of a 96-well plate was coated with FGFR2 recombinant protein by being immersed with 100 μl of 1 μg/ml FGF2-PBS solution for 1 h at room temperature. The wells were washed three times with 200 μl of 0.1% Tween 20-PBS before being blocked with 200 μl of 5% milk-PBS for 2 h. Recombinant human FGFR-Fc (final concentration 1 μg/ml) was premixed with various amounts of anti-FGFR antibodies in 5% milk-PBS for 45 min. The mixtures were transferred to the FGF2-coated multiwell plate (100 μl/well), incubated for 1 h at room temperature, and decanted. The plate was then washed three times with 200 μl of 0.1% Tween 20-PBS. Ligand-bound recombinant FGFR-Fc was probed with an HRP-conjugated anti-human Fc secondary antibody.

FGFR kinase phosphorylation assay. The effects of anti-FGFR1 antibodies on FGF-signaling were determined using cell-based phosphorylation assay. L6 cells expressing full-length human FGFR1c or FGFR1b were cultured in 12-well tissue culture plates with 10% FBS-DMEM. Confluent cells were sequentially serum starved in medium containing 0.1% FBS and 5 μg/ml heparin for 20 h, treated with anti-FGFR1 antibodies for 1 h, exposed to FGF2 for 10 min, and lysed on ice for 30 min. Lysates from FGFR1c-expressing L6 cells were subjected to SDS-PAGE followed by Western blotting. Phosphorylated and total FGFR1c were detected with commercial anti-phosphotyrosine and anti-FGFR1 antibodies, respectively. Lysates from FGFR1b-expressing L6 cells were immunoprecipitated with 50 μg/ml IMC-4H overnight at 4°C to enrich the receptor due to low FGFR1b expression level. The receptor-antibody complex was retrieved using protein A/G beads for 4 h at 4°C and subjected to SDS-PAGE followed by Western probes of the phosphorylated and total FGFR1b, using the commercial antibodies.

Animals

All animal facilities had controlled climate and alternating 12:12-h light-dark cycles. Unless otherwise specified, female C57BL/6 or Nu/Nu C57BL/6 mice (age 6–8 wk; Charles River Laboratories, Wilmington, MA) were housed in groups and given rodent chow (cat no. 5001, 3.3 kcal/g; fat content ~5%; PMI Feeds, St. Louis, MO) and water ad libitum. Adult male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), each weighing 280–300 g, were individually caged and given rodent chow and water ad libitum. Male naive cynomolgus monkeys (age 3–4 yr old; Guangxi Grand Forest Primate, Dayiling Monkey Diet; Animal Specialties, Hubbard, OR). Unconsumed biscuits were removed and counted at the end of each day. Water was provided ad libitum. Cannulated female C57BL/6 mice (3rd ventricle, age 6–10 wk; Charles River Laboratories) were individually caged and given rodent chow and water ad libitum. Routine quality controls were performed by the supplier on the cannulated animals to ensure proper cannulation. All animal procedures conformed to the guidelines of the Department of Agriculture and the National Institutes of Health.
**Weight Loss Experiment**

Mice were weighed and then injected intraperitoneally (ip) or subcutaneously (sc) with antibodies or saline solution three times weekly on Mondays, Wednesdays, and Fridays. At the end of each study, plasma samples were collected, and concentrations of the injected human antibodies were determined using an ELISA in which recombinant human FGFR1b or FGFR1c was the antigen, and the presence of human antibodies in the plasma was detected by a HRP-conjugated anti-human light-chain antibody. To evaluate the possible toxicity of IMC-H7, female Nu/Nu mice were injected ip with 20 mg/kg antibodies on Mondays, Wednesdays, and Fridays for 2 wk and then subjected to serum chemistry evaluation (AST, ALT, CK, BUN, cholesterol, and triglyceride) by Laboratory Corporation of America (Raritan, NJ). After the animals had been killed by CO₂ asphyxiation, the livers, kidneys, hearts, duodena, colons, lungs, stomachs, pancreata, and spleens were harvested and subjected to histopathology evaluation by Experimental Pathology Laboratories (Herndon, VA).

**Food Intake Measurement**

For long-term (15 days) observation of food intake, rats were injected ip with IMC-A1 or control human IgG on day 1. The quantities of available food and water were determined every 24 h and replenished if necessary. Daily intakes of food or water were calculated and recorded.

For short-term continuous monitoring of food intake, rats were adapted to an automatic feeding system (57) and accustomed to handling for 7 days before the experiment. On test day, food was removed 1 h before treatment. Rats were then randomly divided into two treatment groups, injected ip with 10 mg/kg IMC-A1 or control hIgG, and given access to chow 2 h after the injection. Data were recorded at 1-min intervals with WinWedge Pro (Tal Technologies, Philadelphia, PA) and averaged every 30 min throughout the dark and following light cycles.

**Aversion Tests**

Conditioned taste aversion test. The conditioned taste aversion (CTA) test has been used to test the involvement of general malaise or illness in drug-induced reduction of appetite (2, 55). To get the rats used to drinking saccharin, 18-h water-deprived animals were given brief access to saccharin solution (0.01% in distilled water). Four days later, 18-h water-deprived rats were given access to saccharin for 20 min, immediately followed by an ip injection of LiCl (2% body weight, 0.15 M), saline, IMC-A1 (10 mg/kg), or human IgG (10 mg/kg). Two days after conditioning, preference for saccharin over water was determined in two-bottle choice tests over two consecutive 24-h periods. The preference was determined by the formula: \[(\text{saccharin intake})/(\text{saccharin intake + water intake})\] × 100.

Kaolin ingestion test. Pica behavior is the increased consumption of nonfood solid substances (such as clay) observed when animals are thought to be ill (33, 37, 38, 56). The kaolin ingestion test is designed to show that pica may be associated with drug-induced sickness in animals. Rats were habituated to full access to kaolin pellets (Research Diets, New Brunswick, NJ) in their home cages for 7 days before testing. On test day, rats were randomly divided into four treatment groups and received an ip injection of LiCl (2% body weight 0.15 M), saline (the control for LiCl), IMC-A1 (10 mg/kg) or control hIgG (10 mg/kg). Rats were then returned to their home cage and given access to preweighed kaolin pellets. Chow and water were provided ad libitum. Kaolin intake was measured at 4- and 24-h time points. Kaolin ingested (corrected for spillage) was measured at a 4-h time point.

Need-induced salt intake test. Sickness may lead to a reduction in salt appetite, and thus need-induced salt intake test is often used to evaluate the toxicity of an agent (33, 56). A sodium deficit in rats was achieved through salt deprivation combined with furosemide-induced diuresis. Rats were acclimated for 7 days with full access to salt (0.5 M NaCl solution), water, and regular chow. The salt solution was then removed from the cage and regular chow replaced by sodium-deficient chow (Harlan Teklad, Madison, WI). Twenty-four hours later, rats were weighed, injected sc twice with furosamide (5 mg/kg, 1-h interval), and weighed again 2 h after the second injection. Animals with confirmed diuresis by showing >18 g of weight loss in this 3-h period were selected and deprived of salt for an additional 18 h. Sodium-deficient chow and water were provided ad libitum. These animals were then injected ip with LiCl (2% body weight 0.15 M), saline (the control for LiCl), IMCl-A (10 mg/kg), or control hIgG (10 mg/kg) and subsequently provided with water, salt solution, and sodium-deficient chow at the same time. Consumption of both fluids was recorded at 30-min and 4- and 24-h time points.

**Measurement of Metabolic Indexes**

Mice were individually housed in OxyMax metabolic cages connected to VO₂/CO₂ sensors for oxygen consumption measurements (Columbus Instruments International, Columbus, OH). These cages have arrays of infrared light beams for monitoring locomotor activity. Body weights were measured daily; gas concentrations and activities were measured continuously except when mice were being injected ip with IMC-A1 or a control antibody (nonspecific hIgG). Energy expenditure (kcal/h) and respiratory exchange ratio (RER, also termed respiratory quotient, or RQ) were calculated based on the ratio VO₂/CO₂ with the software provided by Columbus Instruments. Body composition of the animals was measured using a Bruker Minispec Mouse Analyzer (Bruker Optics, The Woodlands, TX).

**Pair-Feeding Experiment**

From the study of Measurement of Metabolic Indexes, the average daily chow consumption of IMC-A1-treated mice was determined to be 2.0 ± 0.1 g under ad libitum conditions. In the pair-feeding experiment, both control antibody- and IMC-A1-treated mice were given 2.0 g of chow per day during the entire course of the study. Mice were subjected to the same treatment regimen and analyses as described in Measurement of Metabolic Indexes.

**Plasma Hormone Measurements in Monkeys**

Blood samples of restrained, un-sedated monkeys were collected by venipuncture of the cephalic veins once during acclimation and once after 3 wk of IMC-A1 treatment (25 mg/kg iv once weekly). Plasma and serum samples were prepared. α-Melanocyte-stimulating hormone (MSH), neuropeptide Y (NPY), insulin, glucagon, leptin, peripheral hormone peptide YY3–36 (PYY3–36), glucagon-like peptide (GLP), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH) concentrations were determined by AniLytics (Gaithersburg, MD); adrenocorticotropic hormone (ACTH) and cortisol levels were determined by SNBL USA. Clinical observations of the animals were performed twice daily (in the AM and PM). Detailed physical exams were conducted weekly. All animals appeared normal during the study period. Necropsy was performed at the termination of the study. No clinical or pathological abnormalities were apparent as a result of the antibody treatments.

**Central Effects**

Semiquantitative RT-PCR. Naïve female Nu/Nu C57/BL6 mice were killed by CO₂ asphyxiation. Total RNA preparations of hypothalamus, median eminence, and pituitary were made using a PureLink Total RNA Purification System (Invitrogen, Carlsbad, CA), followed by treatment with DNase I (amplification grade; Ambion, Austin, TX) to remove any genomic DNA contamination. Semiquantitative PCR (end point assay; SuperArray Bioscience, Frederick, MD) of FGFR1 was performed using primer sets (0.4 µM) that are
specific to b-splice form only (sense: 5'-gaaccttcgaggtatatac-3';
anti-sense: 5'-ggttggctcaggcgttgg-3'); c-splice form only (sense:
5'-gactctggtatataac-3'; anti-sense: 5'-ctcctagcttcagca-3'), or both
b- and c-splice forms (Mouse Fgfr1 RT'PCR primer set, Super-
Array). PCR cycles started with a 30-s denaturing step at 95°C, a 30-s
annealing step at 60°C (55°C for b-splice form-specific primers), and
a 30-s extension step at 72°C. PCR products were resolved on a 2% agarose gel, stained with SYBR SAFE DNA stain (Invitrogen, Carls-
bad, CA), and quantified using Spot Densitometry (Alpha InnoTech System Software, San Leandro, CA).

Immunohistochemistry. Mice were injected ip with IMC-A1 or
C225, killed by CO2 asphyxiation 24 h later, and perfused with a
fixing solution of 2% paraformaldehyde and 15% picric acid. Brains
were removed, fixed for an additional 1.5 h, and treated with 30% sucrose overnight prior to embedding in OCT medium. Brains were
then sectioned (6 μm thickness, 60 μm spacing) from the optic chiasm
caudal to the pituitary stalk, spanning the entire third ventricle. These sections were stained for human antibodies with 1 μg/ml HRP-
conjugated anti-human light chain antibody. Spleen sections from the
same animals were stained under the same conditions to show non-
specific diffusion of the antibodies across blood vessels.

Intracerebroventricular injection. Third-ventricle-cannulated mice
were acclimated for 1 wk. Mice were randomized into two groups and
infused with 3 μg (1 μg/ml) of IMC-A1 or C225. Body weight was
measured immediately before the infusion and 24 h after the infusion.
Food intakes were determined in the 24-h periods before and after the
infusion. Mice were killed by CO2 asphyxiation after completion of the
food intake measurements. Plasma samples were prepared and
human antibody concentrations determined using a sandwich ELISA
in which injected antibodies were captured with immobilized protein
A and detected with the HRP-conjugated anti-human light chain antibody.

In a control experiment, mice were injected once ip with 3-μg antibodies (1 μg/ml). Body weight and plasma human antibody concentration
were determined as described above. Because mice were not individually caged, food intake was not determined.

Lipid Accumulation, Glucose Uptake, and Lipolysis In Vitro Assays
Using Human Adipose Cells

The effects of FGF ligand (FGF2) and anti-FGFR1c antibodies on
adipose cells were examined using glucose uptake, lipid accumula-
tion, and lipolysis assays conducted by Zen-Bio (Research Triangle
Park, NC). Cells were kept in a tissue culture incubator (5% CO2,
37°C) unless otherwise specified. Human subcutaneous preadipocytes
(donated by patients undergoing liposuction and isolated by fraction-
ation centrifugation, Zen-Bio) were cultured in DMEM-Ham’s F-12
(1:1, vol/vol) medium with 15 mM HEPES and 10% FBS. These cells
were seeded onto 96-well plates and allowed to differentiate in
condition medium (100 μM IBMX, or IMC-A1 + 100 μM IBMX in 1% BSA-KRB solution. The conditioned medium (100 μl/well) was collected, and the
glycerol contents were assayed using Free Glycerol Assay Reagent (Chemicon International).

Statistical Analysis

Statistical analyses were conducted using SigmaStat version 2.03
(SPSS, Chicago, IL). Food intake in the automated feeding monitor
was analyzed by repeated-measures ANOVA, comparing the average intake during consecutive 4-h time blocks. For mouse plasma mea
surements, comparisons of treatment groups were analyzed by one-
way ANOVA followed by a Tukey-Kramer test. For the monkey
hormone study, body weights and hormone levels during acclimation
and after IMC-A1 treatment were compared using a paired Student’s
t-test (P = 0.05, two tailed). All other statistical analyses were
performed using Student’s t-tests (P = 0.05, two tailed). All data sets
passed normality tests.

RESULTS

Splice-Form Specificity Of Effects

We isolated anti-FGFR1 antibodies that are splice form-
specific from an Fab phage-display library: IMC-4H recognizes
the b-splice form (FGFR1b), IMC-A1, the c-form (FGFR1c), and IMC-H7, both forms (FGFR1b+c). Each anti-
body binds to both human and mouse receptors with a Kd
between 1 × 10−9 M−1 and 1 × 10−10 M−1 and has no
detectable binding to FGFR2, -3, -4, or other common tyrosine kinase receptors such as EGF, PDGF, or IGF in a BiaCore binding analyses. These antibodies were selected on the basis of their ability to prevent binding of FGF1 or -2 to the soluble forms of the receptors in an ELISA assay (Fig. 1A) and thus to potently inhibit ligand-induced receptor phosphorylation (Fig. 1B). At the concentrations tested, these antibodies did not show any detectable agonist activities. Because IMC-A1 and IMC-H7 both target FGFR1c with almost identical potency, they were used interchangeably (due to availability) in some of the experiments described below, where the difference in the specificity of the two antibodies was not likely to confound the interpretation of the results. The cross-reactivity of these anti-
bodies to rat or monkey receptors has not been characterized. Because FGFR sequences across mammalian species are highly conserved (e.g., >98% identical sequences between mouse and human FGFR1 receptors), and IMC-A1 and IMC-H7 bind to human and mouse receptors with equal affinity, the two antibodies are most likely to cross-react fully with rat and monkey FGFR1c.

Weight Loss

We first tested IMC-A1 and IMC-H7 (4–40 mg/kg, 3
times/wk ip) as anti-cancer agents in male C57BL6 N/Nu
or C3H/HeN mice bearing Du145 xenograft tumors and observed unusual
weight loss in the animals (results not shown). To reproduce the
weight loss effect in naive animals, and to test whether females
would be equally susceptible, we used female C57/BL6 mice in our initial weight loss studies. Mice given ip
injections of 30 mg/kg IMC-A1 or IMC-H7 (3 times/wk)
showed a 35–40% reduction in body weight within 2 wk, but
those treated with IMC-4H did not (Fig. 2A). All three anti-
bodies showed comparable pharmacodynamics in mice and
had plasma concentrations of ~100 μg/ml on the last day of the
study. These results suggest that weight loss resulted from
specific interference with FGFR1c-mediated signaling. We examined dose dependency, reversibility, and route-of-administration effects on antibody-induced weight loss. Female Nu/Nu mice (age 6–8 wk) were used, since their compromised immune system precludes an immune response against the injected human antibody, thus allowing studies of chronic effects of the antibody treatment. Two treatment regimens were used. The first regimen comprised ip (0.19, 1.9, and 19 mg/kg) or sc (0.4, 4, 40 mg/kg) injections of IMC-H7 on days 1, 3, 5, and 8. Sixty days after completion of the first regimen, animals in the ip-injected groups were subjected to the second regimen, in which they were treated at the same doses and frequency (3 times/wk ip) for an additional 24 days (days 69 to 93). The results of the study are shown in Fig. 2B. Weight loss from the first regimen plateaued at 35–40% of the initial body weight, which was achieved at a dose as low as 4 mg/kg (sc). After antibody administration was stopped, all animals recovered their original body weights within time periods (30–45 days) proportional to the doses of antibody. The second regimen caused a similar weight loss plateau (35–40%) despite a considerably longer treatment period, indicating unaltered responsiveness of the animals to the antibody.

**Toxicity Testing**

To evaluate the possible toxicity of the antibody, we injected female Nu/Nu mice ip with IMC-H7 or control nonspecific hIgG 3 times/wk for 2 wk. As shown in Fig. 2B, maximal weight loss was achieved at doses greater than 4 mg/kg. We chose the 20 mg/kg dose in an attempt to magnify any antibody-related adverse effects. One of the seven IMC-H7-treated mice died 2 days after the last injection for unknown reasons (necropsy was not performed). The remaining six animals and the control antibody-treated animals were subjected to serum chemistry analysis and histopathology evaluation 4 days after the last injections. IMC-H7-treated animals (n = 6) lost ~35%...
of body weight but did not show altered plasma levels of ALK, AST, CK, BUN, cholesterol, and triglycerides. Histopathological evaluation of liver, kidney, heart, duodenum, colon, lung, stomach, pancreas, and spleen indicated no IMC-H7 treatment-related abnormalities in these mice (results not shown).

Food Intake

Mice treated with anti-FGFR1c antibody generally consumed less food than the control animals. To quantify the effects of antibody on food as well as water intake, we used individually caged adult male Sprague-Dawley rats (each weighing 280–300 g). Single ip injections of 3 and 30 mg/kg IMC-A1 decreased ad libitum food intake of these rats during the ensuing 24 h by ~36 and 58%, respectively (Fig. 3A). These reductions were largely sustained for 10 days in all animals and were followed by a 5-day gradual reversal in the 3 mg/kg-treated animals but not in the 30 mg/kg-treated ones. Changes in water intake and daily body weight mirrored those of food intake (Fig. 3, B and C), suggesting that these decreases might be secondary to the reductions in food intake. Although no pharmacokinetic analyses of IMC-A1 were performed in rats, the kinetics of food and water intake recoveries was consistent with the antibody half-life of 3–5 days seen in mice.

The effect of IMC-A1 on food intake was also followed more closely during a 24-h period (Fig. 3D). The reduction caused by IMC-A1 became apparent ~10 h after the antibody injection. The cumulative effect of reduced food intake remained significant between hours 14 and 26 (Fig. 3D, P = 0.028–0.015). Over the entire 24-h period, IMC-A1 caused significant weight loss in the animals (Fig. 3D, inset).

Possible Adverse Effects

To assess the possibility of general malaise as a mechanism for the observed reductions in food intake, we subjected rats to three different paradigms for the detection of abnormal behaviors commonly associated with “visceral illness”: CTA, kaolin intake, and need-induced salt intake tests. Visceral illness is a general condition of nausea and anorexia that results from exposure to toxins, infections, etc; this type of illness induces strong taste aversion, geophagia, and/or decreased salt intake (56). LiCl produces strong adverse effects in all three tests (56) and therefore was selected as a positive control in our studies. In the CTA test, LiCl-treated rats almost completely avoided saccharine, whereas IMC-A1-treated rats showed no evidence of aversion to saccharin (Fig. 4A). In the kaolin intake test, LiCl induced significant pica behavior (Fig. 4B). In contrast, IMC-A1 did not induce such behavior. In the salt intake test, only LiCl caused an immediate but transient reduction in salt intake, whereas the other treatments had no effects (Fig. 4C). Although each test is limited in its ability to detect aversive effects from a wide variety of causes, collectively these data strongly suggest that the antibody does not make animals sick (2). Thus, the reduction in food intake occurring 10 h after IMC-A1 injection is unlikely to be a manifestation of illness caused by nonspecific toxicity of the antibody.

Energy Expenditure and Body Composition

We studied energy expenditure and locomotor activity of mice treated with IMC-A1 and control antibody (nonspecific hlgG). For this purpose, female C57BL/6 mice were kept...
IMC-A1 (10 mg/kg, intake test. Rats habituated to kaolin for 7 days received an ip injection of
preference was determined over 2 consecutive 24-h periods. Columns and error

(nonspecific hIgG, 10 mg/kg, n = 5), or IMC-A1 (10 mg/kg, n = 7). Test
preference was determined over 2 consecutive 24-h periods. Columns and error bars are means ± SD. B: IMC-A1 does not induce pica behavior in a kaolin intake test. Rats habituated to kaolin for 7 days received an ip injection of IMC-A1 (10 mg/kg, n = 7), control hIgG (10 mg/kg, n = 5), LiCl (0.15 M, 2% body wt, n = 5), or saline (n = 5). Amount of kaolin chewed was measured 4 and 24 h after rats were given access to kaolin, water, and chow after the injections (*P < 0.001 vs. saline control). Kaolin ingested (corrected for spillage) at 4-h time point was also determined to show the close correlation between kaolin chewed and kaoline ingested. C: IMC-A1 does not affect salt intake in a need-induced salt intake test. Sodium need was induced in rats by salt deprivation in diet coupled with fuorsamide-induced diuresis (5 mg/kg sc). Rats with confirmed diuresis were randomly assigned to 4 groups and administered IMCL-A (10 mg/kg, n = 7), control hIgG (10 mg/kg, n = 5), LiCl (n = 6), or saline (n = 6). Salt (0.5 M NaCl) intake was measured at 30 min, 4 and 24 h afterward. (*P < 0.05 vs. saline control).

individually in metabolic cages for 5 days under either ad libitum feeding or pair-feeding conditions and injected ip with 10 mg/kg IMC-A1 or control antibody on days 2 and 4 (Fig. 5A). Under ad libitum feeding conditions, IMC-A1 decreased body weight as expected. This antibody also decreased energy expenditure, physical activity, and RER (RQ). The reduction in energy expenditure is likely a compensatory response to conserve energy during negative energy balance due to reduced food intake (12, 50, 54). From day 2 to day 5, the period when mice received antibody treatment, the mean RER (RQ) was 0.89 in the control antibody-treated mice vs. 0.83 in IMC-A1-treated animals. This reduction reflects a decrease in using carbohydrate as a source of metabolic energy, and, in conjunction with the hypogagic effect of IMC-A1, may suggest the mobilization of endogenous energy stores in these animals to maintain energy homeostasis. In support of this inference, body composition determinations (by MRI) showed that both fat and muscle masses in these animals were significantly reduced after IMC-A1 treatment (Fig. 5B). When energy expenditure, RER (RQ), and physical activity of these animals were measured again 14 days after cessation of treatment, values from IMC-A1- and control antibody-treated groups were identical (Fig. 5C), indicating that IMC-A1-induced changes in metabolism were transient. Finally, IMC-A1-treated mice and the paired-fed control antibody-treated animals lost the same amount of body weight and had identical rates of energy expenditure, suggesting that reduction in food intake was the primary cause of IMC-A1-induced weight loss (Fig. 5D). Collectively, these data rule out cachexia (which would increase energy expenditure and induce muscle wasting) as the cause of weight loss. Rather, the effect of IMC-A1 is evidently anorexigenic: animals having less interest in food; thus the weight loss, decreased energy expenditure, and increased reliance on endogenous energy stores as fuel. The reduction in food intake was not due to general malaise or illness as indicated by the CTA, kaolin intake, and need-induced salt intake tests. Casual observations of the animals did not reveal any abnormal behaviors caused by the antibody treatment.

**CNS Mediation of IMC-A1 Effects**

If IMC-A1-induced weight loss is caused by reduced food intake, it is likely that the antibody exerts its effect by acting on regulatory circuits in the brain that govern hunger and/or satiety. This possibility is supported by several facts. 1) Neurons in the hypothalamus are known to be involved in control of energy intake (6, 34, 61); 2) FGFR1 is expressed in the hypothalamus, including in neurons of the arcuate nucleus and the median eminence (17); and 3) the median eminence serves as a gateway for blood-borne molecules that participate in the bilateral communication between the brain and peripheral organs. Although the blood-brain barrier generally does not permit large molecules such as antibodies to enter interstitial spaces in the brain, areas of the hypothalamus are among the few CNS sites where this barrier is not present (9). Peptide hormones produced by peripheral organs that signal metabolic status, such as leptin and insulin, must be able to reach hypothalamic regulatory neurons via the blood stream (6). To confirm the presence of FGFR1 and to distinguish its splice form specificity in the hypothalamic region, we first attempted to demonstrate FGFR1 expression in the brain regions using in situ hybridization but failed because of difficulties in designing hybridization probes, which are sensitive as well as specific to only the b- or c-splice form of the receptor. We also tried to demonstrate FGFR1 expression by Western blot using IMC-A1, IMC-4H, and IMC-H7, and our efforts were generally unsuccessful. Western probes of recombinant mouse FGFR1c and FGFR1b using these antibodies were also conducted and worked only when relatively large quantities (>10 ng) of the receptors were present (results not shown). Our data suggest that these neutralizing antibodies do not work well in
Western, presumably due to their sensitivity to any conformational changes of the receptors occurring during the Western. Alternatively, we performed semiquantitative PCR of mouse hypothalamus, median eminence, and pituitary using splice from-specific primer sets with comparable primer efficiency (Fig. 6, A–C). We found that, in all three tissues examined, FGFR1c expression was far more abundant than FGFR1b.

To test the hypothesis that IMC-A1-induced hypophagia is a consequence of CNS activity, we determined whether peripherally administered IMC-A1 can be detected in the hypothalamus and whether antibody administered intracerebrally causes food intake and weight reduction. A fully humanized monoclonal antibody (C225) that recognizes specifically human epidermal growth factor receptor (EGFR) was used as the
control antibody. C225 does not cross-react with mouse EGFR or any mouse proteins homologous to EGFR, such as ErbB2, -3, and -4 (results not shown). This is an important quality because the role of the control antibody in our experiments is to show distribution and activity due to nonspecific interactions with mouse tissues. Female C57BL/6 mice were injected ip with the same amount (10 mg/kg) of IMC-A1 or C225, and distribution of these antibodies in selected mouse tissues was assessed 24 h later using immunohistochemistry. In the brain, intense IMC-A1 staining was detected in the median eminence and the neighboring areas of the mediobasal hypothalamus. C225 did not localize to the same areas (Fig. 6D). In the spleen, the two antibodies stained with similar intensity (Fig. 6E), suggesting that they were present in the blood stream at comparable levels. From these data we conclude that peripherally injected IMC-A1 can reach areas of the hypothalamus relevant to energy intake and inhibit FGFR1c-mediated GFG signaling in constituent neurons.

Fig. 6. IMC-A1 targets FGFR1c receptors in the brain. A: relative efficiency of FGFR1 splice form-specific primers. Purified cDNA vectors containing either mouse FGFR1b or FGFR1c sequence were serially diluted, and RT-PCR (20 cycles) using the splice form-specific primers was performed as described in MATERIALS AND METHODS. B: representative agarose gel image of semiquantitative RT-PCR (30 cycles) of FGFR1. Brain tissues were taken from naive female C57BL/6 mice. To illustrate the specificities of FGFR1 primers, PCR was performed on purified cDNA vectors containing mouse FGFR1b or FGFR1c sequence as well as mouse skin cell cDNA known to contain both sequences. C: relative expression of FGFR1b and FGFR1c in mouse brain tissues. Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPD) PCR product was used as the basis for quantification in all tissue samples. Results are presented as means and ranges (n = 2). D: IMC-A1 was specifically retained in the median eminence and the mediobasal hypothalamus after ip injection. Female C57BL/6 mice (age 6–8 wk, n = 6) were injected ip with 10 mg/kg IMC-A1 or C225, killed 24 h later, perfused, and stained for the presence of hIgG in the hypothalamic region in the brain. Each section is shown at ×4 and ×40 magnifications. E: After ip injection, similar intensity of IMC-A1 and C225 staining was detected in the spleen. Spleen sections of the animals described in D were prepared in parallel with the brain sections. Sections were shown at ×4 magnification.
In an attempt to demonstrate that IMC-A1 has a central effect, C57BL/6 mice were injected icv via the third ventricle with 3 µg of IMC-A1 or C225. Mice receiving IMC-A1 showed a 36.0 ± 4.3% (1.4 ± 0.2 g, mean ± SE) reduction in body weight and lost 5.6 ± 0.5% (1.2 ± 0.1 g) of body weight within 24 h. In contrast, much smaller effects were seen in animals receiving C225 (Fig. 7B). Female C57BL/6 mice (age 6 – 8 wk, 23.9 ± 2.3 g/animal). Plasma samples were obtained 24 h after injections. icv Injections, 3rd-ventricle-cannulated female C57BL/6 mice (age 6 – 8 wk, n = 10); ip injections, female C57BL/6 mice (age 6 – 8 wk, n = 5). Open bars, C225-treated animals; hatched bars, IMC-A1-treated animals; NS, not statistically significant. C: ip injection of 3 µg of IMC-A1 did not cause significant weight loss compared with control. Female C57BL/6 mice (age 6 – 8 wk, n = 5) were injected ip with C225 or IMC-A1. Changes in body weight and food intake 24 h after injection were expressed as %pretreatment values (means ± SE). Open bars, C225-treated animals; hatched bars, IMC-A1-treated animals.

Adipocyte Effects

Because FGF signaling is known to play a role in adipogenesis (14, 23, 43, 51), it is possible that anti-FGFR1c may affect energy homeostasis through an adipocyte-related peripheral mechanism. We tested the effects of IMC-A1 and FGF2 on glucose uptake, lipid accumulation, and lipolysis of human adipocytes in vitro. Neither FGF2 nor IMC-A1 affected any of these activities (results not shown).

Table 1. Body weights and circulating hormone concentrations in monkeys before and after IMC-A1 treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>After</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.3 ± 0.11</td>
<td>2.95 ± 0.09</td>
<td>0.042</td>
</tr>
<tr>
<td>α-Melanocyte-stimulating hormone, pmol/l</td>
<td>14.8 ± 5.1</td>
<td>11.3 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Neuropeptide Y, pmol/l</td>
<td>29.6 ± 12.7</td>
<td>27.5 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>26.9 ± 15.4</td>
<td>32.9 ± 25.3</td>
<td>NS</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>113.3 ± 73.8</td>
<td>144.2 ± 37.5</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.03 ± 0.56</td>
<td>4.76 ± 1.14</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral hormone peptide YY3-36, pg/ml</td>
<td>54.1 ± 12.9</td>
<td>55.0 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Glucagon-like peptide-activity, pmol/l</td>
<td>35.3 ± 48.1</td>
<td>13.0 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Luteinizing hormone, ng/ml</td>
<td>0.38 ± 0.20</td>
<td>0.26 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone, ng/ml</td>
<td>0.45 ± 0.07</td>
<td>0.46 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Adrenocorticotropic hormone, pg/ml</td>
<td>103.5 ± 40.0</td>
<td>49.3 ± 15.8</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>317.0 ± 148.2</td>
<td>283.5 ± 36.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD, n = 3/group. Monkeys were acclimated for 31 days (days 1–31), treated iv with 25 mg/kg IMC-A1 on days 32, 39, and 46. Measurements of body weights and collection of blood samples were conducted early in the morning on days 18 and 54, after the animals were fasted overnight. The animals were killed by exanguination after the 2nd blood collection. Statistical analyses: paired Student’s t-test (P = 0.05, 2-tailed), NS, not significant, P > 0.05.

Neuroendocrine Effects in Monkeys

The hypothalamus links the nervous system to the endocrine system by synthesizing and releasing neurohormones that control the pituitary gland (6, 11, 35, 53, 61). We measured circulating concentrations of some of these hormones in monkeys during acclimation as well as after the animals were treated with IMC-A1 (Table 1). Naive male cynomolgus monkeys (n = 3, age 3–4 yr old) were injected iv with 25 mg/kg IMC-A1 once weekly for 3 wk. Although no pharmacokinetic analyses of IMC-A1 were performed in monkeys, we reasoned, on the basis of allometric scaling (30), that this dosage was mediated through a CNS mechanism. determined due to the fact that not all biscuits taken by the monkeys were eaten. Intake was approximated by subtracting untouched biscuits from the daily biscuit ration. By this calculation, the average food intake posttreatment was decreased by 34% ± 20% compared with that during acclimation (P < 0.05, Student’s t-test).

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DISCUSSION

FGFR1 is widely expressed in the hypothalamus, including neurons in the arcuate nucleus and nerve fibers in the median eminence (17). Here, we show that the receptor is expressed predominantly as the c-splice form in the hypothalamus, the median eminence, and the pituitary. IMC-A1 engages FGFR1c but not FGFR1b, whereas IMC-4H does the opposite. IMC-A1 induced hypophagia, whereas IMC-4H did not. Thus, it is possible that the efficacy of IMC-A1 may be due to this anatomic specificity in brain expression of the c-splice form of the receptor. FGF signaling in the hypothalamic-pituitary axis has been shown to stimulate the secretion of anterior pituitary hormones such as corticotropin-releasing factor, which affects energy homeostasis (17, 35). However, our measurements of circulating hormone concentrations in the monkeys did not show any evidence of altered adenomediulary activity by IMC-A1, suggesting that the effects on energy balance are not mediated via the pituitary axes.

The hypothalamus contains neurons mediating reciprocal influences on energy intake and expenditure, e.g., NPY/agouti-related peptide (AGRP) and POMC/CART (proopiomelanocortin/cocaine- and amphetamine-regulated transcript) (6, 11, 34, 53, 61); it is possible that anti-FGFR1c antibodies may affect body weight by modulating the number, connectivity, and/or activity of these neurons. In addition to its effects on proliferation and differentiation of the neural precursor cells, FGF signaling promotes axon growth and branching (48). Furthermore, FGF signaling plays a role in the regulation of synaptic plasticity. In particular, involvement of FGFR1 in the anatomy of the median eminence is demonstrated by the observation that dominant negative expression of the receptor disrupts axon outgrowth of gonadotropin-releasing hormone neurons and the homing of these neurons to the median eminence (16). Hypothalamic structural plasticity contributes to the control of energy homeostasis (21). For example, leptin replacement in adult ob/ob animals causes “rewiring” of both NPY/AGRP and POMC perikarya (44). Most recently, the long-term effects of ciliary neurotropic factor on body weight have been attributed to hypothalamic neurogenesis (26). Thus, it is possible that IMC-A1 may produce its effects on energy homeostasis by similar central mechanisms, i.e., through affecting hypothalamic neuronal plasticity. Additional studies are needed to ascertain a direct link between FGFR1c-mediated FGF signaling and structural effects in the hypothalamus.

Other regions of the brain, such as the caudal brainstem (i.e., area postrema and solitary tract nucleus), are also known to play a role in feeding behavior/energy expenditure (6, 32, 49) and are also characterized by the reduced integrity of the blood-brain barrier (9). Neurons in these regions integrate peripheral signals (e.g., leptin, insulin, gut hormones) and communicate with the hypothalamus to control energy homeostasis (31). Certain FGF ligands and receptors are expressed in these regions, providing neurotropic signaling (5, 19). The relevance of these systems to the apparent CNS effects of IMC-A1 is not known at this point.

FGF signaling may regulate energy homeostasis through peripheral mechanisms as well. FGF ligands (FGF1, -2, -7, -9, -10, and -18) and receptors (FGFR1, -2, -3, and -4) are expressed in adipose tissue and may play a role in adipogenesis (14, 23, 43, 51). Recently, FGFR19 and -21 have been proposed as novel metabolic regulators in the periphery. It has been shown that FGFR19 increases fatty acid oxidation through FGFR4-mediated inhibition of acetyl-CoA carboxylase-2 and/or stearoyl-CoA desaturase-1 (13, 62); FGF21 increases glucose uptake via upregulation of the GLUT1 glucose transporter in adipocytes (25). Introduction of these ligands, either through systemic injection of exogenous proteins or through transgenesis model, renders the animals resistant to diet-induced obesity (25, 60). The binding of these FGFs to FGFR1c has not been characterized; thus, it is not known whether IMC-A1 affects the signaling of these ligands and therefore modulates these peripheral regulation pathways.

We conducted limited studies of the effects of FGF2 and IMC-A1 on the biology of adipocytes in vitro and found that neither protein altered lipid or glucose metabolism in these cells, suggesting that a peripheral mechanism mediated through adipose tissue is unlikely to contribute significantly to IMC-A1-induced weight loss. It should be noted that our studies of adipocytes were limited to a few cellular activities and experimental conditions and may not reflect closely the situations in vivo. In vivo metabolic studies, however, indicated that weight loss could be accounted for almost entirely by the decrease in food intake, arguing for a predominant central mechanism for weight loss caused by IMC-A1 treatment.

The potential role of FGF signaling in the hypothalamic regulation of energy homeostasis has been noted previously, and FGF1 and -2 have been implicated as possible satiety signals. For example, fasting reduces, and refeeding increases, endogenous hypothalamic FGF expression (mRNA and protein levels), and icv-administered FGF1 or -2 decreases food intake in rats (20, 40). Feeding suppression by FGF1 is associated with upregulation of c-Fos activity in periventricular astrocytes (58) and increased protein kinase C activity in glucose-sensitive neurons of the lateral hypothalamus (41). icv-Administered anti-FGFR1, anti-FGFR2, or anti-FGFR1 polyclonal antibodies increase food intake (28, 52). Our investigation supports the relevance of FGF signaling to energy homeostasis in the hypothalamus, but some of our findings are apparently at odds with previously reported data. In particular, our antibodies that antagonize FGFR1c showed a potent anorexigenic effect. There are a number of possible explanations for this apparent discrepancy. First, both FGF1 and -2 are present in the brain in abundance (17, 7); additional icv injections may result in abnormally high concentrations of the ligands in the brain. The pleiotropic and polytropic nature of FGF signaling suggests that excessive ligand may suppress downstream signaling that is otherwise stimulated by the same ligand at lower concentrations and, more importantly, that FGFRs other than FGFR1c may be involved in mediating FGF signaling (15). Second, it is difficult to interpret the effects of icv-injected polyclonal antibodies, since neither the specificity nor the activity of these polyclonal antibodies has been sufficiently characterized. We used monoclonal antibodies that are specific to FGFR1 splice forms. We showed that these antibodies block FGF2 from binding to the receptor and thus inhibit receptor phosphorylation and that only antibodies antagonizing FGFR1c-mediated signaling were anorexigenic in vivo. These antibodies do not activate the receptors in cell-based phosphorylation assays at concentrations as high as 200 nM. It should be noted that the specific FGF ligand(s) inhibited by IMC-A1 in vivo is unknown. Although FGF1 and -2 are the most likely candidates,

REFERENCES


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