GASTROENTEROLOGY 2007;133:1534–1543

Peptide YY Regulates Bone Turnover in Rodents

KATHERINE E. WORTLEY,* KAREN GARCIA,* HARUKA OKAMOTO,* KAREN THABET,* KEITH D. ANDERSON,* VICTOR SHEN,‡ JIM P. HERMAN,§ DAVID VALENZUELA,* GEORGE D. YANCOPOULOS,* MATTHIAS H. TSCHÖP,§ ANDREW MURPHY,* and MARK W. SLEEMAN*

Regeneron Pharmaceuticals Inc, Tarrytown, New York; ‡MDS Pharma Services, Bothell, Washington; and §Department of Psychiatry, University of Cincinnati, Cincinnati, Ohio

Background & Aims: **Peptide YY (PYY) and pancreatic polypeptide (PPY) are members of the neuropeptide Y peptide family. The neuropeptide Y receptor signaling pathway has been implicated in a number of physiologic processes, including the regulation of energy balance and bone mass. To investigate the contribution of endogenous PYY and PPY to these processes, we generated both *Pyy*- and *Ppy*-deficient mice.** ***Methods:*** **$Pyy^{-/-}$ and $Ppy^{-/-}$ mice and their respective wild-type littermates were studied from 8 weeks to 9 months of age. Food intake, metabolic parameters, and locomotor activity were monitored using indirect calorimetry. Body composition and bone parameters were analyzed using dual energy x-ray absorptiometry, histomorphometry, and vertebral compression testing.** ***Results:*** **Studies in these mice showed an osteopenic phenotype specific to the *Pyy*-deficient line, which included a reduction in trabecular bone mass and a functional deficit in bone strength. Furthermore, female $Pyy^{-/-}$ mice showed a greater sensitivity to ovariectomy-induced bone loss compared with wild-type littermates. No food intake or metabolic phenotype was apparent in male or female $Pyy^{-/-}$ mice on standard chow. However, female $Pyy^{-/-}$ mice on a high-fat diet showed a greater propensity to gain body weight and adiposity. No metabolic or osteopenic phenotype was observed in *Ppy*-deficient mice.** ***Conclusions:*** **These results indicate that endogenous PYY plays a critical role in regulating bone mass. In comparison, its role in regulating body weight is minor and is confined to situations of high-fat feeding.**

Peptide YY (PYY) and pancreatic polypeptide (PPY) are syntenic genes on mouse chromosome 11 that share structural similarity to neuropeptide Y (NPY).[1,2] The neuronal and hormonal functions of PYY and PPY have not been fully characterized and have been hampered by their cleavage into multiple bioactive peptides, all of which circulate in the blood stream and bind with varying affinity to a family of G protein–coupled receptors, Y1–Y6. NPY is one of the most potent orexigenic peptides identified and is widely expressed in the brain.[3] In contrast, PYY and PPY are mainly expressed in peripheral tissues; endocrine L cells have been identified as the major source of circulating PYY, but studies have also documented expression in alpha cells of pancreatic islets and subnuclei of the brainstem.[4–6] PYY secretion is increased following food intake in both humans and rodents, and it binds with similar affinity to all Y receptors.[7] Full-length PYY is cleaved by dipeptidyl peptidase IV to PYY3-36. This peptide constitutes the majority of total PYY in the postprandial state and is reported to be a more selective ligand for the Y2 receptor subtype, although binding and activation of the Y1 and Y5 receptor subtypes are still observed.[8,9] PPY is primarily expressed in pancreatic islets with some expression in the gut.[5] Similar to PYY, PPY secretion increases postprandially but shows selectivity for the Y4 receptor.[10]

Whereas the physiologic effects of NPY are well documented, those of PPY are less clear but reports have been published that include inhibition of pancreatic secretion, gallbladder activity, intestinal motility, and a reduction in food intake following peripheral infusion.[10,11] The physiologic function of PYY, particularly in rodents, is perhaps the most controversial. Initial reports highlighted strong and consistent decreases in food intake and body weight following short-term and long-term administration of this peptide.[12] Many of these effects were, however, not reproducible by other laboratories[13,14] or attributed to a conditioned taste aversion response following short-term administration.[14] In such circumstances, genetic deletion studies are often useful in generating decisive data in regard to the biological function of a gene. Unfortunately, the analysis of the various *Pyy* null mice by different investigators has only added to the confusion. Initial reports documented no changes in food intake and body weight in mice that simultaneously lacked Pyy and Ppy.[15] Analysis of an independent line of mice that specifically lack Pyy showed a sex-specific adult-onset mild obese phenotype without any disruption in food intake.[16] In yet a third line of *Pyy* null mice, a more

Abbreviations used in this paper: BMC, bone mineral content; BMD, bone mineral density; NPY, neuropeptide Y; PPY, pancreatic polypeptide; PYY, peptide YY; WT, wild-type.

0016-5085/07/$32.00
doi:10.1053/j.gastro.2007.08.024

recent study reported morbid early-onset obesity and marked hyperphagia.[17]

In the present study, we generated both *Pyy*- and *Ppy*-deficient mice to investigate the physiologic function of these peptides and surprisingly found an osteopenic phenotype specific to the *Pyy*-deficient line.

Materials and Methods

Generation of $Pyy^{-/-}$ *Mice and* $Ppy^{-/-}$ *Mice and Experimental Procedures*

Mice were generated using the previously described high-throughput homologous recombination technology[18] (Supplementary Information; see supplemental material online at www.gastrojournal.org). All procedures were conducted in compliance with protocols approved by the Regeneron Institutional Animal Care and Use Committee. Animals had free access to either standard chow (5020; Purina, St Louis, MO) or a high-fat diet (45% fat, 93075; Harlan Teklad, Madison, WI) unless otherwise specified.

Body Composition, Indirect Calorimetry, and Food Intake

Body composition, including bone mineral density (BMD) and bone mineral content (BMC), was determined before and after a high-fat diet for each individual animal using dual emission x-ray absorption (PIXImus; Lunar, Madison, WI) and the percentage lean muscle and fat mass calculated. Metabolic measurements were assessed both on standard chow (at 8–10 weeks of age) and after exposure to a high-fat diet using an Oxymax (Columbus Instruments International Corp, Columbus, OH) open circuit indirect calorimetry system as previously described.[19] Food intake on a standard diet was assessed by automated measurements in metabolic cages or on a high-fat diet by cage top weights.

Biochemistry

All biochemical analyses were performed on serum samples. Serum samples were collected either from retroorbital bleeds from mice fasted for 4 hours and briefly anesthetized with isoflurane or from trunk blood collected from decapitated mice fasted overnight. For details on assays used, refer to the Supplementary Information (see supplemental material online at www.gastrojournal.org).

Expression Analysis

β-galactosidase staining and immunohistochemistry were performed on adult mice that had been deeply anesthetized (240 mg/kg ketamine, 48 mg/kg xylazine, intramuscularly) and exsanguinated with ice-cold heparinized saline. Tissue was fixed by transcardial perfusion of 2% paraformaldehyde (for β-galactosidase staining) or 4% paraformaldehyde (for immunohistochemistry) in 0.1 mol/L phosphate buffer, postfixed for 2 hours, and then cryoprotected for at least 24 hours in 2 changes of buffered 30% sucrose at 4°C with agitation before sectioning. Immunohistochemical staining of Pyy and Ppy was performed on slide-mounted sections of pancreas, ileum, and colon (16 μm) and on free-floating coronal sections of brain (40 μm) as described[19,20] using a rabbit serum against porcine/rat Pyy (1:50,000; Bachem, King of Prussia, PA) or a guinea pig serum against rat pancreatic polypeptide (1:5000; Linco, St. Charles, MO). Fluorescently conjugated secondary antisera (Alexa 488 goat anti-rabbit or goat anti–guinea pig; Molecular Probes, Eugene, OR) were used at a dilution of 1:200. To visualize β-galactosidase, sections were incubated in buffered 1 mg/mL X-Gal (Molecular Probes) for 12–24 hours at 37°C as described previously.[21] The sections were counterstained with eosin.

Histology and Histomorphometry of Bone Tissue

For histologic analyses, L3–L5 vertebrae were dissected and collected into 10% buffered formalin. Lumbar vertebrae were dehydrated through a series of ascending ethanol solution, cleared with xylene, infiltrated with methylmethacrylate, and embedded in methylmethacrylate/catalyst. Sagittal sections, through the middle of the vertebral body, were obtained using a rotary microtome (model RM2165; Leica Microsystems Inc, Bannockburn, IL). One 4-μm section was stained with Goldner's trichrome stain, and one 8-μm section was mounted unstained.

Bone histomorphometry was performed using OsteoMeasure image analysis software program (OsteoMetrics, Inc, Atlanta, GA) interfaced with an Eclipse E400 light/epifluorescent microscope and video subsystem (Nikon, Tokyo, Japan). Histomorphometric evaluations of the stained slides were performed in a blinded manner, and the nomenclature and calculations used for all parameters were based on standardized terms and formulae.[22] For measurement of the lumbar vertebral body, the region of interest was composed of an area of marrow cavity that was approximately 0.2 mm from the 2 growth plates and the ventral and dorsal cortex. Evaluation of structural features was performed from an image projected using a 10× objective. Six fields on each slide were evaluated.

Compression Test of Lumbar Vertebral Body

Compression testing of L2 vertebrae was performed using an Instron Mechanical Testing Machine (model 4465 retrofitted to 5500; Instron Corp, Canton, MA). For more details on the methods used, refer to the Supplementary Information (see supplemental material online at www.gastrojournal.org).

Data and Statistical Analyses

Data are expressed as mean ± SEM, and comparisons were performed using a *t* test or repeated-measures analysis of variance where appropriate using the program