Expression of Y2R mRNA Expression in Subcutaneous and Visceral Adipose Tissues Compared between Normal Weight and Obese Female Subjects

Saimai Chatree, Malika Churintaraphan, Nay Chi Nway, Chantacha Sitticharoon, M.D.*
Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Neuropeptide Y (NPY) and Y2 receptor (Y2R) are found to be expressed in adipose tissue. This study aimed to compare Y2R mRNA expression in subcutaneous and visceral fat tissues as well as serum PYY levels between obese and normal weight subjects. We demonstrated that Y2R mRNA in visceral fat was greater ($p<0.05$) in normal weight subjects than in obese subjects with comparable expression in subcutaneous adipose tissue. Y2R mRNA was ($p<0.05$) expressed higher in subcutaneous than visceral fat tissue in obese subjects. Moreover, Y2R mRNA expression in subcutaneous fat tissue was positively correlated to its expression in visceral adipose tissues ($R=0.932$, $p<0.001$). In visceral fat, Y2R expression was negatively correlated with serum NPY levels ($R=-0.415$, $p<0.05$). Serum PYY was similar between obese and normal weight groups. In conclusion, higher Y2R mRNA expression in visceral fat tissue might be involved in lower adiposity. Further investigation regarding activation of Y2R action in visceral adipose tissue may reveal novel strategies for adiposity reduction.

Keywords: Y2R, PYY, NPY, obesity

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INTRODUCTION

Neuropeptide Y, the most potent orexigenic peptide in the brain,[1] is mainly expressed in the hypothalamus, especially in the arcuate nucleus (ARC).[2] NPY acts through a family of G-protein coupled receptors3 with Neuropeptide Y1 receptor (Y1R), Neuropeptide Y2 receptor (Y2R), Neuropeptide Y4 receptor (Y4R), and Neuropeptide Y5 receptor (Y5R) being presented in humans[3]. Y2R is localized in several brain areas in rats[4] and humans especially in the hippocampus, hypothalamus, and brain stem.[4] In the periphery, Y2R is located on the terminals of sympathetic and parasympathetic neurons in rats[5], tongue epithelium, colon in mice,[6] vessels, endothelial cells, preadipocytes of mice and humans and in subcutaneous abdominal fat pads in mice.[7]

Correspondence to: Chantacha Sitticharoon
E-mail: chantacha.sit@mahidol.ac.th
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It is well known that Y2R has equally high affinity to the C-terminus segment of NPY and peptide YY (PYY)[8] with NPY_{1-36} and PYY_{3-36} being the major forms in circulation, respectively.9 PYY, a member of a structurally related peptides family including NPY and pancreatic polypeptide (PP),[10] is released from intestinal L-cells.[11] Binding of the PYY_{3-36} to the hypothalamic Y2R is involved in the induction of satiety[3,9] or reduced weight gain in rats and mice,[12-14] and humans.[15] Furthermore, specific deletion of Y2R at ARC of the hypothalamus increases food intake and body weight.[16] Hypothalamic Y2R mRNA is significantly decreased in diet-resistant (DR) mice[17] and rats[18] when compared to diet-induced obesity (DIO) counterparts. Preadipocyte differentiation of mouse 3T3-L1 preadipocytes is stimulated by NPY action on lipid filling of new adipocytes and this effect is blocked by Y2R antagonist[7] showing 50% reduction of adipose tissue weight and volume in both obese and lean mice. Chronic stress increases Y2R expression in visceral fat in high-fat, high-sugar fed mice, resulting in fat accumulation and metabolic complications which can be prevented or reversed by pharmacological Y2R inhibi-

tion resulting in apoptosis of endothelial and fat cells.[19] Furthermore, Y2R antagonist decreases visceral fat mass in mice[7]. Y2R mRNA was remarkably up-regulated in subcutaneous abdominal fat of obese leptin-deficient (ob/ob) mice compared with controls.[7] On the other hand, another study has reported that Y2R agonist tended to decrease fat mass by 12% in DIO mice.[13]

In conclusion, the central effect of NPY via Y2R appears to reduce food intake and body weight whereas the effect on adipose tissue is still inconclusive. Y2R mRNA expression in human adipose tissue has not been studied. This study aimed to compare Y2R mRNA between obese and normal weight subjects as well as between subcutaneous and visceral adipose tissues. Moreover, comparison of serum PYY levels between obese and normal weight humans was also determined.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Siriraj Institutional Review Board (Si.533/2009) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. All subjects gave informed consent prior to the study. All 30 Thai female patients who underwent abdominal surgery were recruited and divided into 2 groups, which were obese (n=17) (BMI>25 kg/m^2) and normal weight (n=9) (BMI=18.5-22.9 kg/m^2). The other 4 subjects were overweight or lean and were recruited for correlation analysis. Subjects on endocrine therapy (e.g. steroids, hormone replacement therapy, thyroxine), or those who were pregnant, lactating, undergoing traumatic operations, had malignant diseases, or who underwent operations related to endocrine diseases or severe abdominal inflammation were excluded. In this study, male subjects could not be recruited because most male patients who underwent open abdominal surgery had cancer or emergency operations which fell into the exclusion criteria. Other studies collecting visceral adipose tissue from open abdominal surgery were also done in females.[20, 21] The phase of menstrual cycle of female subjects recruited in this study could not be controlled because most subjects had myoma uteri and were presenting with irregular menstruation.

Demographic details

Age, body weight, and BMI were collected from subjects.

Blood and tissue collection

Blood was collected during the fasting state before operations. Abdominal subcutaneous and omental (visceral) adipose tissues were collected during operations. Four to five pieces of 0.5 cm of each type of adipose tissues, which are abdominal subcutaneous and visceral adipose tissues, were collected from each subject. Adipose tissues were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Analysis of Y2R mRNA expression in adipose tissues

The total RNA was isolated using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. One µg of total RNA was reverse transcribed to complementary DNA (cDNA) using iScript cDNA Synthesis Kit (Bio-RAD, Hercules, California, USA). To quantify Y2R mRNA expression, real-time reverse transcription polymerase chain reaction (RT-PCR) was done using the reagents and protocol contained in the VeriQuest SYBR Green qPCR Master Mix (Affymetrix, Santa Clara, California, USA). Low density lipoprotein receptor-related protein 10 (LRP-10) was used as a reference gene because it is the most stably expressed gene in human adipose tissue.[22] Primer sequences were designed by the authors with exon to exon sequences and were blasted to confirm primer specificity using published nucleotide sequences from PubMed database as shown in Table 1. The PCR amplification was performed under the following conditions: Taq DNA polymerase activation at 95ºC for 10 min, 40 cycles of DNA denaturing at 95ºC for 15 sec, annealing at 57ºC for 60 sec and extension at 72ºC for 30 sec. For every RT reaction, no template control (NTC) was performed as a negative control and human brain tissues were used as a positive control. The actual RT-PCR product size was proven using DNA ladder (BioLabs Inc, Ipswich, Massachusetts, England) by electrophoresis. The 2-ΔCT method was applied as a comparative method of quantification.

Analysis of serum PYY

PYY serum levels were analyzed by a commercial enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, Burlingame, California, USA). The range of PYY detection was 0-100 ng/ml and the minimum detectable concentration was 0.1ng/ml. The absorbance O.D. was read at 450 nm by Synergy HT Multi-Detection Microplate Readers (BioTek Instruments, Inc., Winooski, VT, U.S.). Intra-assay coefficients of variances were 5.69%. A commercial kit for blood measurement of neuropeptide Y receptor including Y2R is not available and a study regarding determination of blood Y2R levels or its soluble form has not been reported.

Statistics

Kolomonov-Smirnov test was performed to test normality. For normal distribution, comparison between obese and normal weight groups was performed by Unpaired t-Test and comparison between subcutaneous and visceral adipose tissues was done by Paired t-Test. Comparison of data with non-normal distribution was performed with non-parametric test. Data were presented as mean±S.E.M. Correlation coefficients were calculated using 2-tailed Pearson product-moment correlation. A p-value less than 0.05 was considered as statistical significance.

RESULTS

Demographic data of subjects and serum PYY levels

Range and median of age and comparisons between obese and normal weight subjects including body weight and serum PYY levels are shown in Table 1.

TABLE 1. The real-time PCR primer sequences and product size of Y2R and LRP-10.

Genes	Sequence	Product size (base pair)
Y2R-forward	5'-GGCCTACTGCTCCATCATCTTG-3'	228
Y2R-reverse	5'-CCCTGGGCATAGGGCACC-3'	
LRP-10-forward	5'-GATGGAGGCTGAGATTGTGCA-3'	169
LRP-10-reverse	5'-TGGAGTCATATCCTGGCGTAAG-3'	

TABLE 2. Demographic data of subjects and serum PYY levels compared between obese and normal weight subjects. **p<0.01, ***p<0.001 compared with normal weight group.

Parameters	Obese (n=17)	Normal weight (n=9)
Age (range), year	37-58	28-51
Age (median), year	46	43
Body weight, kg (mean±S.E.M.)	74.36±3.90***	51.67±2.05
Serum PYY, ng/ml (mean±S.E.M.)	0.55±0.02	0.52±0.06

Y2R mRNA expression in adipose tissue

Y2R mRNA expression was greater in the normal weight group than in the obese group in visceral adipose tissues ($p<0.05$) (Fig 1A), but not in subcutaneous adipose tissue. Y2R mRNA expression was significantly higher in subcutaneous adipose tissue than in visceral adipose tissues in obese subjects ($p<0.05$), whereas its expression was not different in normal weight and overall subjects (Fig 1B).

Correlations between 2 factors

A strong positive correlation was presented between the expression of Y2R mRNA in subcutaneous and visceral adipose tissues ($R=0.932$, $R2=0.869$, $p<0.001$). Y2R expression in visceral fat was inversely correlated to serum NPY levels ($R=-0.415$, $R2=0.172$, $p<0.05$).

DISCUSSION

This study focused on the comparison of Y2R mRNA expression in subcutaneous and visceral adipose tissues as well as serum PYY levels between normal weight and obese subjects. In this study, Y2R mRNA was detected in human adipose tissue and was 4.1 fold significantly higher in a normal weight group than an obese group in visceral fat tissue. The result might indicate that higher expression of Y2R mRNA in normal weight subjects in visceral adipose tissue might be involved in decreased adiposity. Furthermore, a negative correlation between serum NPY and visceral Y2R mRNA was found. Since NPY increases fat proliferation and differentiation[7] and a previous study in our laboratory showed higher serum NPY levels in obese when compare to normal weight subjects.[23] All of these evidences might suggest that high Y2R mRNA expression in visceral fat might be closely related to a reduction in adiposity. These results were inconsistent with a previous study showing that Y2R antagonist decreased visceral fat mass in mice.[7] The discrepancy of these results might be partly described by the distinction of species. Y2R mRNA in subcutaneous fat was comparable between obese and normal weight subjects suggesting that the effect of NPY via Y2R might not differ between obese and normal weight humans. Our result was inconsistent with a previous study reporting that Y2R mRNA was up-regulated in subcutaneous abdominal fat of obese leptin-deficient (ob/ob) mice.[7] The possible explanation might be due to distinctions of species and

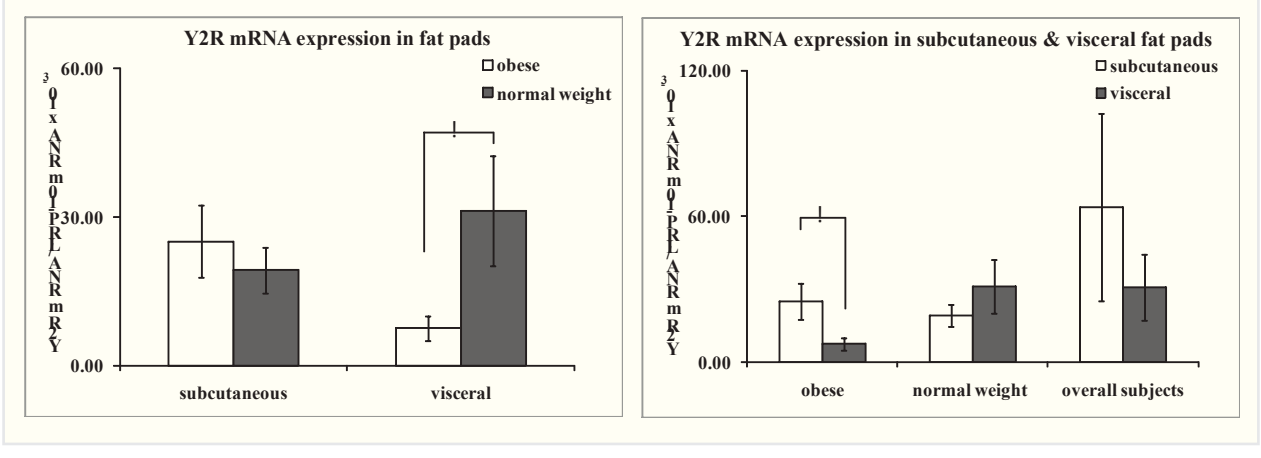

Fig 1. Mean (±S.E.M.) Y2R mRNA expression normalized to LRP-10 (reference gene) in subcutaneous and visceral adipose tissues. Panel A shows Y2R mRNA expression compared between obese and normal weight subjects in subcutaneous and visceral fat pads. Panel B presents Y2R mRNA expression compared between subcutaneous and visceral fat pads in obese, normal weight, and overall subjects. *p<0.05 compared between groups.

leptin deficiency status. It has appeared that peripheral function of Y2R in fat accumulation might be different between subcutaneous and visceral adipose tissue and this issue needs to be further investigated.

Y2R mRNA expression was 3.3 fold higher in subcutaneous adipose tissue when compared to visceral adipose tissue in obese, but not in other groups indicating that Y2R in obese humans was more capable of being synthesized in subcutaneous than in visceral fat tissue. A previous study in human showed that the fat cell volume of obese subjects was greater than non-obese subjects[24] with subcutaneous adipocyte size being larger than omental adipocyte.[25] Thus, it is possible that Y2R was more synthesized in subcutaneous than visceral adipose tissue resulting from the abilities of large adipocytes. Furthermore, a strong positive correlation between Y2R mRNA expression in subcutaneous and visceral adipose tissue was found suggesting a concordant action of this receptor in 2 depots.

Serum PYY levels were not different between obese and normal weight subjects which was consistent with a previous study in American volunteers showing that fasting PYY levels were similar between obese and normal weight groups.[26] However, it has been revealed that fasting PYY levels were significantly lower in obese children than lean children.[27] This different finding might be caused by differences in age, group of comparison, and BMI cut-off points. Although fasting PYY levels were similar between groups, it has been reported that postprandial PYY response was blunted in obese people causing low satiety signalling.[28]

In conclusion, this study highlighted higher Y2R expressions in visceral adipose tissue in obese compared to normal weight subjects and a negative correlation of visceral Y2R mRNA and serum NPY which might indicate up-regulated visceral Y2R in lower adiposity status. Further studies are required to disclose the effect of NPY via Y2R on fat accumulation, especially in human visceral adipose tissue.

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