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Peptide tyrosine tyrosine (PYY) as a new strategy for treating obesity

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Background
Obesity is a risk factor for obesity-related disorders such as type 2 diabetes mellitus, vascular disease, osteoarthritis, sleep apnea, and malignancy. Obesity cohinder the individual work capacity, and the cost for managing obesity complications is high.
Objective
The objective of this research was to study the role of pancreatic polypeptide family including neuropeptide Y and peptide tyrosine tyrosine (PYY) in obesity development and its metabolic changes.
Materials and methods
Twenty-seven adult female albino rats of a local strain were randomized into three equal groups for 5 weeks: sham-operated group, ovariectomized nontreated group, and ovariectomized treated group received $PYY_{3–36}$ at a dose of 50 μg/kg, by intraperitoneal injection twice daily during the fifth week.
Results
Peripheral $PYY_{3–36}$ administration reduces food intake, body weight gain, and serum glucose in ovariectomized obese female rats.
Conclusion
PYY system may offer a new therapeutic strategy for obesity management and its metabolic abnormalities.

Keywords:
metabolic disease, obesity, ovariectomized rats, pancreatic polypeptides, peptide tyrosine tyrosine

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Introduction

Lifestyle modification strategies (diet and exercise) currently form the main treatments for obesity. However, results are generally disappointing, and the majority of people who attempt lifestyle modification regain any lost weight within 5 years [1]. Accumulation of excess body fat (and hence development of obesity) occurs when energy intake exceeds energy expenditure in the long term [2]. Our progressive understanding of the physiological mechanisms of appetite regulation should help with the development of future pharmacological approaches to combat obesity [3].

Pancreatic polypeptide family includes neuropeptide tyrosine (NPY), peptide tyrosine tyrosine (PYY), and pancreatic polypeptide. Its name derives from the single-letter code (Y) for the amino acid tyrosine, as it contains several tyrosine residues. These peptides are structurally and biologically similar, but they are synthesized and secreted from different sources [4].

PYY is released from the L cells of the gastrointestinal tract, with increasing tissue concentrations found in the distal portions [5]. In the circulation, it exists in two major forms: $PYY_{1–36}$ and $PYY_{3–36}$. $PYY_{1–36}$ is rapidly proteolyzed by enzyme dipeptidyl-peptidase IV. The cleaved product, $PYY_{3–36}$, is bioactive. PYY is able to cross the blood–brain barrier (BBB) by transmembrane diffusion from the circulation [5].

PYY is also considered an appetite-regulating hormone given that its secretion reduces hunger and imparts satiety [6].

Obesity-related disorders such as type 2 diabetes, vascular diseases, osteoarthritis, hazards of bariatric surgery, and the economic burden in treating obesity complications led us to find another strategy for treating obesity. Therefore, we aimed to investi- gate the effect of intraperitoneal injection of a potent Y_2 receptor agonist $PYY_{3–36}$ on ovariectomy (OVX)-induced obesity and its metabolic abnormalities.

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Materials and methods

This study was carried out in the Medical Physiology Department, Faculty of Medicine, Sohag University. This was approved by Research Ethics Committee considering care and use of laboratory animals in Sohag University.

Animals and groups

Twenty-seven adult albino rats of local strain, of body weight ranging between 150 and 200 g at the beginning of this study, were used. The age of these rats was about 8–10 weeks at the start of the experimental work. They were housed in groups in cages (20 cm width×32 cm length×20 cm height) at room temperature with natural light/dark cycles for 1 week to acclimatize them to lab conditions. Rats were fed a standard diet of commercial rat chow (El-Gomhoria Company, Cairo, Egypt) and tap water ad libitum until the time of the experiment [6]. During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. The rats were divided into three equal groups: (a) control sham-operated group, (b) ovariectomized nontreated group, and (c) ovariectomized treated group. The animals were accustomed to the injection procedure by intraperitoneal injection with saline (0.5 ml/rat) for 2 days before $PYY_{3–36}$ administration [7]. Next, each rat received $PYY_{3–36}$ (Sigma Aldrich, 2033 Westport Center Dr., St. Louis, MO 63146, USA) at a dose level of 50 μg/kg, by intraperitoneal injection twice daily during the fifth week. It was prepared by dissolving $PYY_{3–36}$ in saline solution [8].

Ovariectomy [9]

Lee index was used to determine obesity in rats using body weight and nasoanal length. It was measured at the beginning of the study, at the end of the fourth week, and at the end of the fifth week. Lee index was calculated for each rat according to the following formula: Cube root of body weight (g)×10/nasoanal length (mm) [10]. Obesity was considered when the Lee index was greater than 0.3. At the end of the fifth week, rats were killed after an overnight fast by decapitation, and blood samples were collected, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge; Andreas Hettich GmbH & Co. KG, Föhrenstr 12, D-78532 Tuttlingen, Germany). Serum was then withdrawn into identified Eppendorf tubes and stored at −20°C until the determination of hypothalamic NPY concentration [11], total serum cholesterol [12], LDL cholesterol (LDL-C) [13], HDL cholesterol (HDL-C) [14], serum glucose [15], and serum leptin [16].

Statistical analysis

Statistical analysis was performed using the computer software program prism (Comshare's version of a decision support system), version 3.3. Data were expressed as mean±SE. Analysis of variance with Bonferroni's multiple comparison test was used to find intergroup significance. P value less than 0.05 was considered statistically significant.

Results

OVX caused a significantly higher body weight from the second week until the end of the study, as compared with the sham-operated control group. Injection of $PYY_{3–36}$ caused a significantly lower body weight as compared with the OVX group, as shown in Table 1.

Lee index changes in the different groups

In OVX groups, rats also had a Lee index higher than 0.3 at the end of the fourth and fifth weeks and were

Table 1 Body weight changes in the different groups

Body weight (g)	Groups		
	Sham-operated control	OVX	OVXT
Initial	189.7±2.3	187.7±3.5	188.7±1.7
After 1 week	200.7±3.2	190.5±6.9	193±1.2
Change from initial (%)	5.7	1.49	2.27
After 2 weeks	213.2±2	220.5±1.04[a]	221.2±1.7[a]
Change from 1 week (%)	6.1	15.7	14.6
After 3 weeks	216.7±1.1	231.2±1.4[a]	233±1.2[a]
Change from 2 weeks (%)	1.6	4.8	5.3
After 4 weeks	218.5±0.6	237.2±2.05[a]	240.2±0.6[a]
Change from 3 weeks (%)	0.83	2.5	3.09
After 5 weeks (treatment week)	219.7±0.4[b]	241±0.9[a,b]	233.5±0.2[a,c,b]
Change from 4 weeks (%)	0.54	1.6	−2.7

Data are expressed as mean±SE of nine rats in each group. OVX, ovariectomized; OVXT, ovariectomized treated with $PYY_{3–36}$ during the fifth week; PYY, peptide tyrosine tyrosine. [a]Significant from the sham-operated control group. [b]Final value significant from its corresponding initial value. [c]Significant from the OVX group.

considered obese. The Lee index was significantly higher in the OVX group as compared with the control group. Injection of $PYY_{3–36}$ during the fifth week did not decrease the Lee index below 0.3 and rats were still obese, but the Lee index in the OVXT group was significantly lower as compared with the OVX group and insignificant as compared with the sham-operated control group, as shown in Table 2.

Time-course changes in daily food intake in the different studied groups

In ovariectomized groups, food intake was significantly lower during the first week and then significantly higher until the end of the study as compared with the control group. Injection of $PYY_{3–36}$ caused a significantly lower food intake as compared with the sham-operated control and OVX groups (Table 3).

The changes in weight of the gastrocolic omentum fat in the different studied groups

In the OVX group, the weight of the gastrocolic omentum fat (GCOF) was significantly higher as compared with the control group. Injection of $PYY_{3–36}$ caused a significantly lower weight of the GCOF as compared with the OVX group, as shown in Table 4; however, it was still significantly higher than the sham-operated control group.

Serum glucose, leptin, and hypothalamic neuropeptide Y concentrations in the different studied groups

Serum glucose level was significantly higher in the ovariectomized groups as compared with the corresponding control groups. On the other hand, injection of $PYY_{3–36}$ caused a significantly lower serum glucose level in OVX as compared with nontreated groups; however, the levels were still significantly higher than the control groups (Table 5).

Serum leptin was significantly higher in the ovariectomized groups as compared with the corresponding control groups. Injection of $PYY_{3–36}$ caused a significantly lower serum leptin level in OVX groups as compared with noninjected groups, but the levels remained significantly higher than the control groups (Table 6).

It was observed that the hypothalamic NPY was significantly lower in high-fat diet groups as compared with control groups. On the other hand, NPY was significantly higher in the OVX group as compared with the sham-operated control group. Injection of

Table 2 The Lee index in the different groups

Lee index	Groups		
	Sham-operated control	OVX	OVXT
Initial	0.294±0.001	0.293±0.001	0.29±0.002
End of the fourth week	0.295±0.001	0.315±0.002[a]	0.308±0.001[a]
End of the fifth week	0.296±0.001	0.316±0.002[a]	0.3002±0.0001[b]

Data are expressed as mean±SE of nine rats in each group. OVX, ovariectomized; OVXT, ovariectomized treated with $PYY_{3–36}$ during the fifth week; PYY, peptide tyrosine tyrosine. [a]Significant from the sham-operated control group. [b]Significant from the OVX group.

Table 3 Daily food intake changes in the different groups

Food intake (g/day)	Groups		
	Sham-operated control	OVX	OVXT
After 1 week	13.2±0.31	12.05±0.24[a]	12.08±0.29[a]
Change from control (%)	–	–8.7	–8.4
Change from OVX (%)	–	–	0.2
After 2 weeks	12.07±0.22	20.8±0.51[a]	21.2±0.75[a]
Change from control (%)	–	72.3	75.6
Change from OVX (%)	–	–	1.9
After 3 weeks	13.04±0.44	20.9±0.56[a]	20.7±0.44[a]
Change from control (%)	–	60.2	58.7
Change from OVX (%)	–	–	–0.9
After 4 weeks	13.18±0.31	20.5±0.3[a]	21.2±0.4[a]
Change from control (%)	–	55.5	60.8
Change from OVX (%)	–	–	3.4
After 5 weeks (treatment week)	13.21±0.37	20.5±0.32[a]	10±0.31[a,b]
Change from control (%)	–	55.3	–24.2
Change from OVX (%)	–	–	–51.2

Data are expressed as mean±SEM of nine rats in each group. OVX, ovariectomized; OVXT, ovariectomized treated with $PYY_{3–36}$ during the fifth week; PYY, peptide tyrosine tyrosine. [a]Significant from the sham-operated control group. [b]Significant from the OVX group.

Table 4 The weight of GCOF in different groups

	Groups		
	Sham-operated control	OVX	OVXT
Weight of GCOF (g)	2.15±0.06	5.22±0.19[a]	2.88±0.05[a,b]
Difference from control (%)	–	142.7	33.9
Difference from OVX (%)	–	–	–44.8

Data are expressed as mean±SEM of nine rats in each group. GCOF, gastrocolic omentum fat; OVX, ovariectomized; OVXT, ovariectomized treated with $PYY_{3–36}$ during the fifth week; PYY, peptide tyrosine tyrosine. [a]Significant from the sham-operated control group. [b]Significant from the OVX group.

Table 5 Serum glucose, serum leptin, and hypothalamic NPY in the different groups

Parameters	Groups		
	Sham-operated control	OVX	OVXT
Serum glucose (mg/dl)	71.3±1.4	124.2±0.9[a]	113.6±3.6[a,b]
Change to control (%)	–	74.1	59.3
Change to OVX (%)	–	–	–8.5
Serum leptin (ng/ml)	5.4±0.1	27.07±1.4[a]	13.21±0.6[a,b]
Change to control (%)	–	401.2	144.6
Change to OVX (%)	–	–	–51.2
Hypothalamic NPY (pg/mg)	16.1±0.8	22.5±1.4[a]	11.9±0.7[a,b]
Change to control (%)	–	–39.7	–26.08
Change to OVX (%)	–	–	–47.1

Data are expressed as mean±SEM of nine rats in each group. OVX, ovariectomized; OVXT, ovariectomized treated with Peptide $YY_{3–36}$ during the fifth week. [a]Significant from the sham-operated control group. [b]Significant from the OVX group.

Table 6 Serum level of lipid profile in ovariectomized groups

	Groups		
	Sham-operated control	OVX	OVXT
TC (mg/dl)	134.3±1.7	158.6±3.04[a]	157.6±2.9[a]
Change from control (%)	–	18.08	17.3
Change from OVX (%)	–	–	0.6
TGs (mg/dl)	95.08±0.9	91.3±1.09[a]	89.9±3.1[a]
Change from control (%)	–	–4.14	–5.7
Change from OVX (%)	–	–	–1.5
HDL-C (mg/dl)	55.5±0.6	39.3±0.1[a]	39.2±0.2[a]
Change from control (%)	–	–29.1	–29.3
Change from OVX (%)	–	–	–0.2
LDL-C (mg/dl)	66.2±0.6	73.05±1.4[a]	71.1±1.2[a]
Change from control (%)	–	10.3	7.4
Change from OVX (%)	–	–	–2.6

Data are expressed as mean±SEM of nine rats in each group. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; OVX, ovariectomized; OVXT, ovariectomized treated with $PYY_{3–36}$ during the fifth week; PYY, peptide tyrosine tyrosine; TC, total cholesterol; TG, triglyceride. [a]Significant from the sham-operated control group.

$PYY_{3–36}$ caused a significantly lower hypothalamic NPY level in both OVX and high-fat diet groups as compared with both control and nontreated groups.

Serum level of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol in different studied groups

In ovariectomized groups, in comparison with the sham-operated control group, the serum levels of total cholesterol and LDL-C were significantly higher associated with a significant lower serum levels of triglycerides (TGs) and HDL-C. Injection of $PYY_{3–36}$ to ovariectomized rats caused insignificant differences on serum lipid profile as compared with the OVX group.

Discussion

Results of the previous studies that have examined the effect of $PYY_{3–36}$ administration on food intake and body weight regulation were contradictory. Peripheral administration of $PYY_{3–36}$ reduced food intake and decreased body weight gain in both humans and rodents. The anorectic effect of peripheral $PYY_{3–36}$

may be mediated via the presynaptic inhibitory Y_2 receptor present on arcuate NPY neurons [5]. Intravenous PYY infusion has been found to decrease energy intake and reduce hunger in healthy individuals [7] and those with obesity [17]. It has been reported that fasting and postprandial PYY levels are lower in those with obesity and higher in normal-weight individuals [18]. Another study observed that acute and chronic administration of $PYY_{3–36}$ in rats either increased or did not change food intake and body weight [19].

In the present study, induction of obesity was performed by OVX. The occurrence of obesity was confirmed by the Lee index, and a Lee index of 0.3 or more indicated obesity [20]. In experimental rats of this study, it was higher than 0.3. After induction of obesity, rats were treated by intraperitoneal administration of $PYY_{3–36}$ during the fifth week with continuous standard rat chow diet in OVX groups.

OVX caused a significantly higher body weight from the second week until the end of the study as compared with the control group. The correlated increase in body weight and food intake found in the present study following OVX is in agreement with Abdel-Hakim *et al.* [21], Zhang *et al.* [22], and Pósa *et al.* [23] who explained the increase in body weight by the increase in food intake. Another possible mechanism of increased body weight is the increased lipogenesis and decreased lipolysis, which was evidenced in the present work by a significant increase in Lee index and weight of GCOF. This is in agreement with Abdel Razek *et al.* [24].

The present results revealed that OVX was accompanied with significantly lower food intake during the first week and then increased significantly until the end of the study as compared with the control group. There were several possibilities that explained the mechanism of lowered food intake following OVX. Stressors such as surgical trauma, anesthesia, and manipulation of internal viscera may result in this reduction [25]. Stengel *et al.* [26] found that surgical stress resulted in activation of the brain corticotropin-releasing factor (CRF) receptors. In the brain, CRF is well established to activate sympathetic outflow while reducing gastric vagal activity. A certain study supported the role of this brain stress pathway by the blockade of delayed gastric emptying immediately after surgery in mice lacking the CRF1 receptor [27].

The mechanism of increased food intake after OVX may be because of the increased hypothalamic NPY concentration, as found in the present study and by Jiang *et al.* [28] and Zhang *et al.* [29] The increased hypothalamic NPY concentration may be because of the lack of estrogen hormone, as found by Rivera *et al.* [30] and Santollo *et al.* [31]. Estrogen has been proposed to act directly and indirectly to decrease NPY release and to decrease food intake. Previous study showed colocalization of estradiol and NPY immunoreactivity in some neurons in the ARC nucleus, which suggests a direct genomic modulation of NPY neurosecretion by estrogens in the hypothalamus [32]. Another study detected that estrogen decreased the expression of NPY in the ARC nucleus [33] and decreased NPY release in the paraventricular nucleus through estrogen receptors, which were expressed by NPY neurons in the hypothalamus [34].

In OVX rats, the weight of GCOF was significantly higher as compared with the control group [21,35]. There are several mechanisms underlying the visceral fat accumulation in OVX rats. OVX-induced hyperphagia resulted in excess accumulation of fat, as found by Abdel-Hakim *et al.* [21] It may also be because of cessation of endogenous estrogen production [36]. Estrogen has direct effects on abdominal adipocytes to stimulate lipolysis through higher lipoprotein lipase activity, and the opposite occurs in subcutaneous fat. Thus, loss of subcutaneous fat and accumulation of visceral fat occur after OVX [24,37].

In the present study, it was found that OVX produced a significantly higher serum level of total cholesterol and LDL-C and a significantly lower serum level of TGs and HDL-C. Similar results were reported by Elbassuoni *et al.* [38] and El Habachi *et al.* [36]. These disturbances in lipid profile could be attributed to the greater visceral fat accumulation, and this was evident in the present study by greater GCOF weight. The reduction of HDL-C and TGs after OVX might be related to estrogen withdrawal. Estrogen decreases HDL catabolism through reduction of hepatic lipase activity [39] and increases TG biosynthesis through activation of glucose-6-phosphate dehydrogenase, which is an indicator of the glucose flux through the pentose phosphate pathway producing nicotinamide adenine dinucleotide phosphate hydrogen [40].

Peripheral administration of $PYY_{3–36}$ after induction of obesity in OVX rats significantly lowered food intake, body weight, Lee index, GCOF weight, hypothalamic NPY, serum leptin, and glucose, as compared with control groups. It is possible that the body weight and Lee index reduction with peripheral administration of PYY is primarily driven by reductions in food intake and weight of GCOF. This is in agreement with Reidelberger *et al.* [41] and Mittapalli and Roberts [42].

The reduction of food intake with $PYY_{3–36}$ administration in the ovariectomized group is in agreement with the study by Papadimitriou *et al.* [43] Within the central nervous system, PYY exerts its anorectic effects via actions in the ARC nucleus of the hypothalamus. The ARC is in close proximity to the deficient BBB of the median eminence of the hypothalamus, thus allowing this region to respond rapidly to the released gut hormones in the circulation, including PYY, which is able to cross the BBB by transmembrane diffusion from the circulation [44,45]. Evidence confirmed that PYY_{3-36} exerted the inhibition on food intake in a Y_2-dependent manner, as Y_2 receptors are abundantly expressed on NPY neurons in the ARC of the hypothalamus. Another study reported that the anorexigenic actions of PYY were abolished in Y_2 knockout mice and blocked by Y_2 antagonist [42]. Y_2 receptors primarily act as presynaptic autoreceptors modulating endogenous NPY release. In particular, PYY inhibits NPY neurons and reduces hypothalamic NPY mRNA and/or protein content as found in the present results and by Yulyaningsih *et al.* [46].

A certain study observed that acute and chronic administration of $PYY_{3–36}$ in rats could not produce any effect on food intake and body weight [47], whereas another study reported that peripheral administration of $PYY_{3–36}$ increased food intake and body weight [19]. Challis *et al.* [48] observed the short-term anorectic effects, but none after 7 days of administration on either cumulative weight gain or food intake. Several possibilities may cause inconsistency in the effect of $PYY_{3–36}$ administration on food intake. One possibility may be caused by different experimental protocols or animal strains studied [49]. Another possibility is that insufficient acclimatization to handling and injection of animals results in a stress-induced reduction in appetite and a subsequent failure of $PYY_{3–36}$ to further reduce food intake [50]. The broad distribution of the Y receptor subtypes both centrally and peripherally with the antagonistic effects of stimulation of the different subtypes and the fact that PYY could act both centrally and peripherally when peripherally administered may explain this discrepancy.

Several possibilities explain the reduction of visceral fat with $PYY_{3–36}$ administration. Adams *et al.* [51] and Chelikani *et al.* [52] found that this effect may be secondary to reduced food intake, and/or reflect a direct action of $PYY_{3–36}$ on fat-mobilizing or fat-utilizing tissues, as diminished food intake in response to $PYY_{3–36}$ treatment may lower the insulin: glucagon ratio, increase lipolysis, and decrease de novo lipogenesis.

The reduction in the body weight and GCOF weight may be the cause of significantly lower serum level of leptin with $PYY3_{3–36}$ administration as compared with control groups [53,54]. However, another research documented that $PYY_{3–36}$ produced an insignificant effect on serum leptin [55].

Insulin resistance (IR) can be defined as a state of reduced responsiveness to normal circulating levels of insulin; it plays a major role in the development of type 2 diabetes [56]. There are multiple mechanisms involved in the development of IR, including the following: excess lipid accumulation and dietary fatty acid might be involved in altering the cell membrane composition, thereby impeding the binding of insulin to its receptor [57], inflammatory response, and altered cytokine production from expanded adipose tissue and subsequent paracrine/autocrine-mediated cellular IR [58], mitochondrial dysfunction, and consequently dysfunction of mitochondrial fatty acid oxidative capacity [59]. Previous results suggested that hypothalamic NPY neurons modulate the inhibitory effect of insulin on glucose production via efferent sympathetic nerves innervating the liver and caused IR [60,61]. Therefore, the hypoglycemic effect of peripherally administered $PYY_{3–36}$ as found in the present study and by Chandarana *et al.* [62] may be secondary to reduction of food intake, Lee index, body weight, GCOF weight, and NPY release. All these factors may improve insulin sensitivity and glucose disposal [63].

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Conflicts of interest
There are no conflicts of interest.

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