Endokrynologia Polska
DOI 10.5603/EP.2014.0007
Tom/Volume 65; Numer/Number 1/2014
ISSN 0423–104X

Enhanced food intake by progesterone-treated female rats is related to changes in neuropeptide genes expression in hypothalamus

Zwiększone spożycie pokarmu jest związane ze zmianami ekspresji genów neuropeptydów w podwzgórzu samic szczura po podaniu progesteronu

Ewa Stelmańska[1], Elżbieta Sucajtys-Szulc[2]

[1]Department of Biochemistry Medical University of Gdansk, Poland
[2]Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk, Poland

Abstract

Introduction: Progesterone-treated females eat more food, but the mechanism underlying this effect is not well understood. The aim of the study was to analyse the effect of progesterone on neuropeptide genes expression in rat hypothalamus.
Material and methods: Experiments were carried out on female and male Wistar rats. Animals were treated with progesterone (100 mg per rat) for 28 days. NPY and CART mRNA levels in hypothalamus were quantified by real-time PCR. The serum progesterone concentration was determined by radioimmunoassay.
Results: Progesterone administration to females caused an increase in food intake, body mass, and white adipose tissue mass. Elevated circulating progesterone concentration up-regulated NPY and down-regulated CART genes expression in hypothalamus of females. In males, elevated blood progesterone concentration had no effect on food intake, body and fat mass and on the neuropeptide genes expression in hypothalamus. Moreover, administration of progesterone in females resulted in decrease of PR mRNA level in hypothalamus. No effect of progesterone administration on PR mRNA level in hypothalamus of males was found.
Conclusions: The changes in neuropeptide genes expression in hypothalamus may lead to stimulation of appetite and might explain the observed increase in food intake, body and adipose tissue mass in progesterone-treated females. **(Endokrynol Pol 2014; 65 (1): 46–53)**

Key words: *progesterone; cocaine and amphetamine regulated transcript (CART); neuropeptide Y (NPY); progesterone receptor (PR)*

Streszczenie

Wstęp: Podanie progesteronu samicom szczura prowadzi do wzrostu ilości spożywanego przez nie pokarmu, jednak mechanizmy odpowiedzialne za ten efekt nie są do końca znane. Celem tej pracy było sprawdzenie, czy obserwowane pod wpływem progesteronu zmiany w ilości spożywanego pokarmu przez szczury mogą być związane ze zmianami ekspresji genów neuropeptydów w podwzgórzu.
Materiał i metody: Doświadczenie zostało przeprowadzone na samicach i samcach szczura rasy Wistar. Po 28 dniach po podaniu progesteronu (100 mg/szczura) pobrano podwzgórza i zmierzono poziom mRNA NPY i CART w oparciu o metodę PCR w czasie rzeczywistym. Stężenie progesteronu w surowicy krwi badanych zwierząt oznaczono metodą radioimmunologiczną.
Wyniki: Podanie progesteronu prowadzi do wzrostu masy ciała, tkanki tłuszczowej i ilości spożywanego pokarmu u samic szczura. Zmianom tym towarzyszy wzrost ekspresji genu NPY i obniżenie ekspresji genu CART w podwzgórzu. U samców podanie progesteronu nie ma wpływu na masę ciała, masę tkanki tłuszczowej i ilość spożywanego pokarmu oraz ekspresję genów neuropeptydów w podwzgórzu. Ponadto wykazano, że podanie progesteronu samicom szczura prowadzi do obniżenia ekspresji genu receptora progesteronowego w podwzgórzu. Zmian takich nie zaobserwowano u samców.
Wnioski: Zmiany ekspresji genów neuropeptydów w podwzgórzu samic szczura, którym podano progesteron, mogą prowadzić do stymulacji apetytu, co w konsekwencji wpływa na wzrost ilości spożywanego pokarmu, zwiększenie masy ciała i masy tkanki tłuszczowej. **(Endokrynol Pol 2014; 65 (1): 46–53)**

Słowa kluczowe: *progesteron; transkrypt regulowany przez kokainę i amfetaminę (CART); neuropeptyd Y (NPY); receptor progesteronowy (PR)*

This work was supported by the Medical University of Gdansk (grant ST-41).

Introduction

Progesterone regulates various functions in the body related not only to reproduction or ovulation [1]. Some data suggests that progesterone may affect carbohydrates and lipids metabolism, organism growth, body composition and food intake [2, 3]. Studies on rodents have demonstrated that progesterone treatment increases body weight and adipose tissue mass by regulation of the expression of specific genes [4]. It is well known that in pregnancy (upon high concentration of progesterone in blood) the most important regulatory

Ewa Stelmańska Ph.D., Department of Biochemistry, Medical University of Gdansk, Debinki St. 1, 80–211 Gdańsk, Poland, tel./fax: +48 58 349 14 65, e-mail: bori@gumed.edu.pl

process is the increase in appetite and food intake. It has also been observed that progesterone-treated animals eat more food [2, 5, 6], but the mechanism of progesterone action on food intake is still elusive. Under physiological conditions, across the ovarian cycle, female rats eat up to 25% less during the night of ovulation and oestrus (the day after progesterone release). However, this is the effect of oestradiol not progesterone. Ovariectomy eliminates cycle and increases daily food intake. Oestradiol treatment reverses these effects, but progesterone treatment has no such effect [7]. Only a pharmacological dose of progesterone can reverse the inhibitory effect of oestradiol on eating in rats [8]. In women, enhancement of eating during luteal phase has been attributed to increased plasma progesterone, but there is little support for this suggestion. Moreover, some studies indicate that a pharmacological dose of progesterone had no effect on eating in women [9]. It is worth noting that a synthetic progesterone derivative (17α-OH-progesterone derivative) — megestrol acetate — is being used to stimulate appetite and to induce weight gain in patients with cancer or AIDS, as well as in cachectic elderly subjects [10, 11].

It has been observed that progesterone-treated animals eat more food, but the mechanisms underlying these effects are not well understood. It is commonly known that hypothalamic orexigenic (like NPY) and anorexigenic (i.e. CART) neuropeptides play an important role in the regulation of appetite and body weight homeostasis. Under physiological conditions, various factors are involved in the regulation of neuropeptide genes expression [12, 13]. Moreover, some studies have indicated that food restriction, fasting or refeeding affect neuropeptide genes expression in hypothalamus [14–16].

The influence of progesterone on neuropeptide genes expression is not clearly defined. It has been shown that progesterone penetrates to different regions of the brain, including the hypothalamus [17]. The presence of progesterone receptor (PR) in hypothalamus was also proven [18]. Progesterone has been reported to increase NPY content of the hypothalamus in oestrogen-primed [19] and oestrogen-primed ovariectomised rats [20, 21]. The expression of neuropeptide genes in hypothalamus upon high level of progesterone in rats is different. Some data shows a significant increase of NPY and AgRP mRNA levels, and decrease of POMC mRNA level in the arcuate nucleus of pregnant rats [6]. In pseudopregnant rats, despite hyperphagia and the high level of progesterone, hypothalamic levels of the same neuropeptides were found unchanged [6].

We have recently shown that progesterone influences metabolic and endocrine functions of white adipose tissue in female rats by regulating some genes expression [4, 5]. We observed that progesterone-treated females, in contrast to males, eat more food than controls.

Based on these observations, and data from the literature, we hypothesised that progesterone may influence the food intake by regulation of neuropeptide genes expression in hypothalamus. The aim of this study was to analyse the effect of progesterone on NPY and CART genes expression in rat hypothalamus.

Material and methods

Animals and treatment

Ten-week-old female and male Wistar rats, initially weighing approximately 200 g and 260 g, respectively, were used. The rats were assigned to four groups: control females (n = 10), females treated with progesterone (n = 10), control males (n = 10) and males treated with progesterone (n = 10). Under general anaesthesia induced by intraperitoneal injection of ketamine (60 mg/kg) and xylasine (6 mg/kg), a pharmacological dose (100 mg per rat) of progesterone (Sigma-Aldrich, St. Louis, MO, USA) was implanted subcutaneously in the lower abdomen. Control animals underwent an identical sham-operation. Males and females were housed in individual wire-mesh cages, in the same room at 22°C, under a light:dark 12h:12h cycle with lights on at 7:00 a.m. The animals were allowed free access to tap water and food (the commercial diet composition was described in [22]). The amount of consumed food was measured every other day. The animals were killed by decapitation 28 days after the progesterone implantation. Hypothalamus (taken as a whole) was collected (as described in [23]), rapidly frozen in liquid nitrogen and stored at –80°C for subsequent analyses. Blood was collected from the neck artery, centrifuged, and the separated serum was stored at –80°C until progesterone concentration was measured. All procedures involving the animals were conducted in agreement with our institutional guidelines for the care and use of laboratory animals.

Determination of serum progesterone concentration

Serum progesterone concentration was measured by radioimmunoassay using a commercial immunoassay kit, according to the manufacturer's instructions (Institute of Atomic Energy POLATOM, Radioisotope Centre, Poland).

RNA isolation

Total cellular RNA was extracted from frozen tissue by a guanidinium isothiocyanate/phenol/chloroform method [24]. The RNA concentration was determined from the absorbance at 260 nm. All samples had 260/280 nm absorbance ratio of approximately 2.0.

Table I. *Serum progesterone concentration in control and progesterone-treated rats (ng/mL). Data is presented as mean ± SEM; number of animals per group (n) = 10*

Tabela I. *Stężenie progesteronu (ng/ml) w surowicy krwi szczurów kontrolnych i otrzymujących progesteron. Wyniki przedstawiają średnią ± SEM; liczba zwierząt w grupie (n) = 10*

Gender	Control	Progesterone-treated	Fold change	Statistical significance
Females	31.3 ± 6.7	76.8 ± 8.4	2.5	$p < 0.01$
Males	1.8 ± 0.5	29.2 ± 7.3	16	$p < 0.01$

cDNA synthesis

First strand cDNA was synthesised from 4 μg of total RNA (RevertAidTM First Strand cDNA Synthesis Kit — Fermentas UAB, Lithuania). Prior to amplification of cDNA, each RNA sample was treated with RNase-free DNase I (Fermentas UAB, Lithuania) at 37°C for 30 min.

Determination of neuropeptides and progesterone receptor mRNA levels by real-time PCR

NPY, CART and PR mRNA levels were quantified by real-time PCR using Chromo4 Real Time Detection System (Bio-Rad Laboratories, Inc, Hercules, CA, USA). The primers were designed with Sequence Analysis software package (Informagen, Newington, USA) from gene sequence obtained from Ensembl Genome Browser (www.ensembl.org). The following oligonucleotide primers pairs were used: 5′-TAGGTAACAAACGAATGGGG-3′ (sense) and 5′-AGGATGAGTGAGATGTGGG-3′(antisense) for NPY; 5′-CTCAAGAGTAAACGCATTCC-3′(sense) and 5′-ACAAGCACTTCAAGAGGAAA-3′ (antisense) for CART; 5′-TCAACCAACTAGGCGAGAGG-3′ (sense) and 5′- ACACCATCAGGCTCATCCA-3′(antisense) for progesterone receptor; 5′-TGTCACCAACTGACGATA-3′ (sense) and 5′-GGGGTGTTGAAGGTCTCAAA-3′(antisense) for β-actin; 5′-CTGAGCACTGGGGAGAAAGGA-3′ and 5′-GAAGTCACCACCACTGCACA-3′ for cyclophilin-A. Real-time PCR amplification was performed in a 20 μl volume using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Each reaction contained cDNA and 0.3 μM of each primer. Samples were incubated for an initial denaturation and polymerase activation at 95°C for 5 min followed by 35 PCR cycles of amplification (92°C for 20 s, 57°C for 20 s, and 72°C for 40 s). Control reactions, with omission of the RT step or with no template cDNA added, were performed with each assay. All samples were run in triplicate. To compensate for variations in the amount of added RNA, and for the efficiency of the reverse transcription, β-actin mRNA was quantified in corresponding samples and the results were normalised to these values. It should be noted that similar results using β-actin and cyclophilin-A as housekeeping genes were found (not shown). Relative quantities of transcripts were calculated using the $2^{-\Delta\Delta CT}$ formula [25]. The results are expressed in arbitrary units, with one unit being the mean mRNA level determined in the control group. Amplification of specific transcripts was further confirmed by obtaining the melting curve profiles and subjecting the amplification products to agarose gel electrophoresis.

Statistics

The statistical significance of differences between the groups was assessed by a one-way analysis of variance (ANOVA) and Tukey's post-hoc test used for further determination of significance of differences. Sigma Stat software (Sigma Stat Inc.) was used. Differences between the groups were considered significant when $p < 0.05$. All data is presented as means of values ($n = 10$) ± standard error of mean (± SEM).

Database Sequence Analysis

The TRANSFAC database (BIOBASE, Beverly, MA, USA) was searched using AliBaba 2.1 (BIOBASE) for putative PR binding sites on the 5′ flanking sequence (2,000 bp upstream and 300 bp downstream from the ATG start codon) of the NPY and CART genes.

Results

Administration of progesterone in females resulted in an approximately 2.5-fold increase of serum progesterone concentration (Table I). As expected, food intake was increased in treated females and remained elevated for most of the treatment period (Fig. 1A). Statistically significant differences in food intake between control and progesterone treated animals were found from the eighth day of the treatment. Food intake by progesterone-treated and control males was essentially similar (Fig. 1B). At the end of the experiment, the gain of body weight in treated females was about 18 g larger than in control females (Fig. 2). No significant changes in the body weight gain of males (despite several-fold increase of serum progesterone concentration [Table I]) were found (Fig. 2). The mass of adipose tissue (the sum of the inguinal, retroperitoneal and parametrial WAT) of rats treated with progesterone, increased substantially only in females (Fig. 3). No effect of progesterone administration on WAT mass in males was

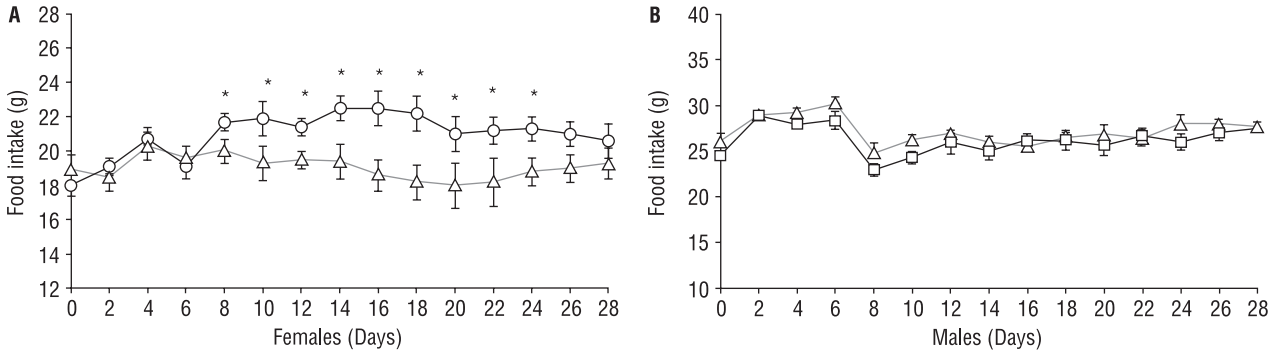

Figure 1. *Daily food intake by control and progesterone-treated females (**A**) and males (**B**). Each point represents the mean ± SEM of ten animals. *p < 0.05 control female rats versus progesterone-treated female rats. (Δ) control rats (females or males respectively); (○) progesterone-treated females; (□) progesterone-treated males*

Rycina 1. *Ilość spożywanego pokarmu w ciągu jednego dnia przez samice (**A**) i samce (**B**) szczurów kontrolnych i szczurów otrzymujących progesteron. Każdy punkt przedstawia średnią ± SEM (n = 10). *p < 0,05 samice kontrolne w porównaniu do samic badanych. (Δ) szczury kontrolne (samice lub samce); (○) samice, którym podano progesteron; (□) samce, którym podano progesteron*

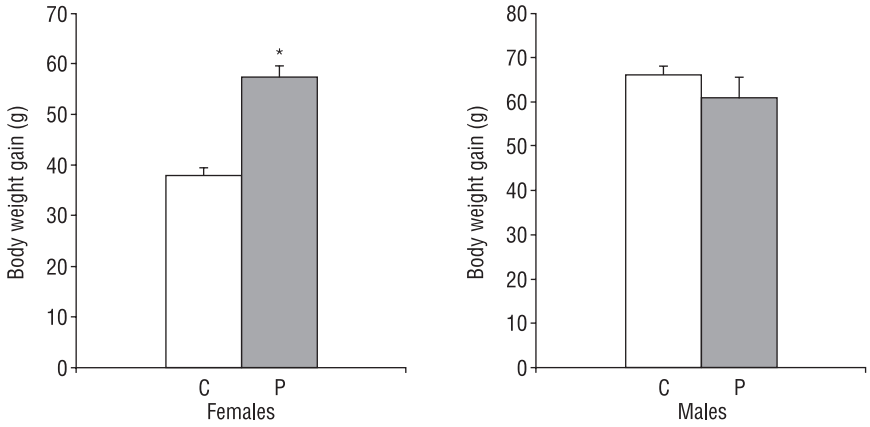

Figure 2. *The body weight gain of control (C) and progesterone-treated rats (P). Data is presented as mean ± SEM (n = 10). *p < 0.01 control female rats versus progesterone-treated female rats*

Rycina 2. *Przyrost masy ciała szczurów kontrolnych (C) i szczurów, którym podano progesteron (P). Wyniki przedstawiają średnią ± SEM (n = 10). *p < 0,01 samice kontrolne w porównaniu z samicami badanymi (P)*

found (Fig. 3). In order to check whether the increase of food consumption in treated females is associated with changes in gene expression of orexigenic and anorexigenic neuropeptides, we measured NPY and CART mRNA levels in hypothalamus. Figure 4 shows that an elevated circulating progesterone concentration was associated with an approximately two-fold increase of NPY mRNA level in hypothalamus of females. The increase in orexigenic neuropeptide mRNA level by progesterone was associated with a significant decrease of anorexigenic neuropeptide mRNA level in hypothalamus. Figure 5 shows that CART mRNA level was approximately three-fold lower in hypothalamus of treated females than in control females. Despite the several-fold increase of circulating progesterone concentration in progesterone-treated males, no significant changes in NPY and CART mRNA levels were found in hypothalamus of males (Figs. 4 and 5). It is worth noting that the serum concentration of progesterone in progesterone-treated males reached the similar value observed in non-treated control females (Table I). Collectively, these results indicate that progesterone administration caused an increase in food consumption of females, associated with an increase of NPY, and a decrease of CART mRNA levels in hypothalamus.

We also examined the effect of progesterone's administration on the PR gene expression in hypothalamus of females and males. As shown in Figure 6, administration of progesterone in females resulted in about a four-fold decrease of PR mRNA level in hypothalamus. No effect of progesterone administration on PR mRNA level in hypothalamus of males has been found (Fig. 6).

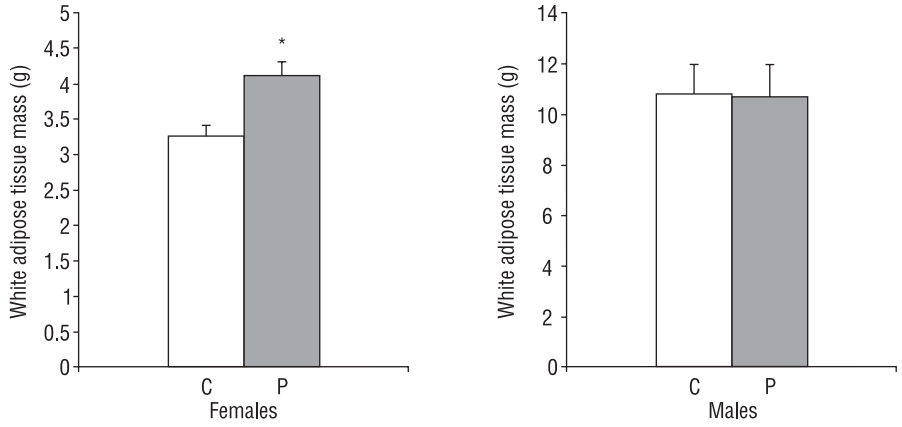

Figure 3. *White adipose tissue mass of control (C) and progesterone-treated (P) rats. Data is presented as mean ± SEM (n = 10). *p < 0.01 control female rats versus progesterone-treated female rats*

Rycina 3. *Masa białej tkanki tłuszczowej szczurów kontrolnych (C) i szczurów otrzymujących progesteron (P). Wyniki przedstawiają średnią ± SEM (n = 10). *p < 0,01 samice kontrolne w porównaniu z samicami badanymi (P)*

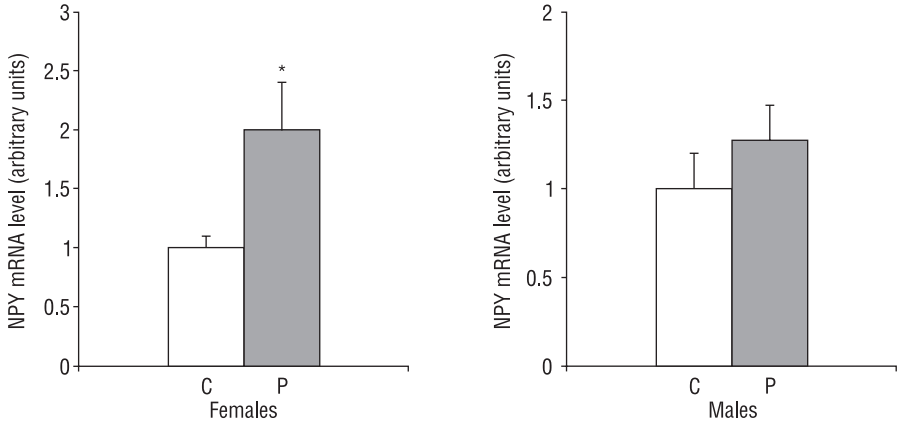

Figure 4. *NPY mRNA level in hypothalamus of control (C) and progesterone-treated (P) rats. Data is presented as mean ± SEM (n = 10). *p < 0.05 control female rats versus progesterone-treated female rats*

Rycina 4. *Poziom mRNA neuropeptydu Y w podwzgórzu szczurów kontrolnych (C) i szczurów, którym podano progesteron (P). Wyniki przedstawiają średnią ± SEM (n = 10). *p < 0,05 samice kontrolne w porównaniu z samicami badanymi (P)*

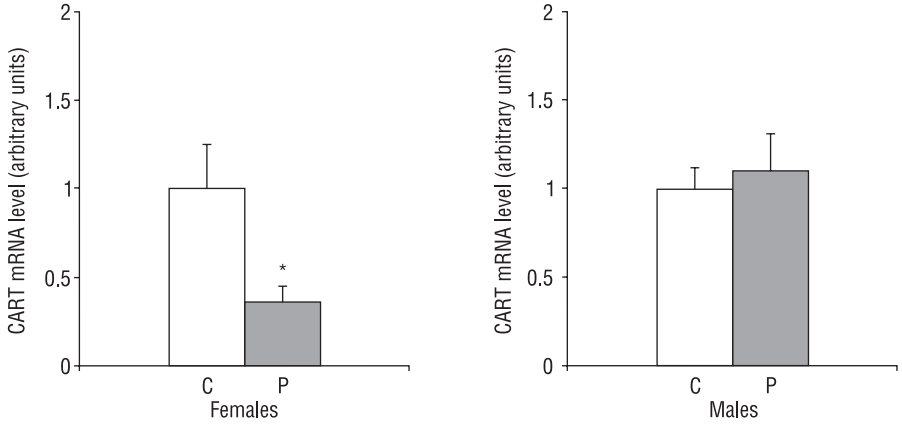

Figure 5. *CART mRNA level in hypothalamus of control (C) and progesterone-treated (P) rats. Data is presented as mean ± SEM (n = 10). *p < 0.01 control female rats versus progesterone-treated female rats*

Rycina 5. *Poziom mRNA CART w podwzgórzu szczurów kontrolnych (C) i szczurów, którym podano progesteron (P). Wyniki przedstawiają średnią ± SEM (n = 10). *p < 0,01 samice kontrolne w porównaniu z samicami badanymi (P)*

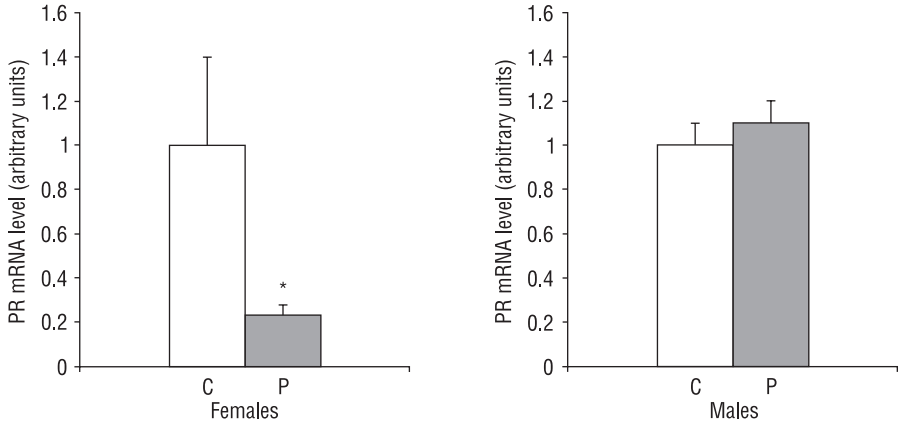

Figure 6. *PR mRNA level in hypothalamus of control (C) and progesterone-treated (P) rats. Data is presented as mean ± SEM (n = 10). *p < 0.01 control female rats versus progesterone-treated female rats*

Rycina 6. *Poziom mRNA receptora progesteronu w podwzgórzu szczurów kontrolnych (C) i szczurów, którym podano progesteron (P). Wyniki przedstawiają średnią ± SEM (n = 10). *p < 0,01 samice kontrolne w porównaniu z samicami badanymi (P)*

Discussion

We examined the effect of progesterone on NPY and CART mRNA levels in hypothalamus, food intake, body weight and adiposity. The changes in the parameters were observed only in females. These animals, after hormone treatment, showed a higher increase in body weight and adipose tissue mass. Our recently published results indicate that the increase of white adipose tissue mass is associated with hypertrophy of inguinal adipocytes [5]. Starting from the eighth day of the experiment, the females examined ate more than the control animals (average about 2 grams per day). In this paper, we observe that long-term treatment of females with progesterone leads to a significant increase in NPY and a decrease in CART mRNA levels in the hypothalamus respectively. These changes could explain the observed increase in food intake in the females. Our results are inconsistent with the data presented by Trujillo et al. [6]. They demonstrated that, despite hyperphagia, NPY mRNA level in hypothalamus was unchanged in pseudopregnant rats [6]. However, it should be emphasised that the serum progesterone concentration of these animals was three times lower compared to our results and reached the value characteristic for the control rats in our experiment.

It seems that the level of circulating hormone may be a decisive factor for progesterone's actions in the tissue. Different strains of rats used in these experiments could also be the reason for different results. The regulation of the expression of these neuropeptides during gestation is still controversial. Most of the results indicate that in the hypothalamus of pregnant rats NPY gene expression is up-regulated [6, 26, 27]. Little is known about the influence of steroid hormones on CART gene expression. Studies in monkeys showed that CART protein level in midbrain CART neurons was unchanged by progesterone administration [28].

To the best of our knowledge, we showed for the first time that CART gene expression is down-regulated by elevated concentration of circulating progesterone in female rats. It is also noteworthy that the POMC mRNA level (another anorexigenic peptide) is lower in pregnant rats [6]. However, other results indicate that the level of POMC mRNA does not change during pregnancy [27].

The mechanism leading to changes in neuropeptide genes expression is not clear. The sequence analysis of the promoter regions of NPY and CART genes predicted one and two PR binding site respectively (see Material and Methods). Thus, we consider that progesterone may directly regulate the NPY and CART genes expression. There is also a possibility that progesterone may act indirectly, affecting the expression of genes involved in regulation of neuropeptides levels. The most important factor regulating hypothalamic neuropeptides mRNA abundance is circulating leptin [29, 30]. We have shown previously that the concentration of plasma leptin in females treated with progesterone does not change despite an increase in leptin gene expression in inguinal adipose tissue [5]. We also observed no changes in serum concentrations of the other adipokines (such as adiponectin) or steroid hormones (cortisol and aldosterone) in progesterone treated animals (data not shown).

It is therefore likely that the observed changes are a direct effect of progesterone's action, especially that progesterone can enter the hypothalamus. Another example of the action of progesterone in the hypothalamus is change in the progesterone receptor gene expression of progesterone treated females. Our data indicates for the first time that PR mRNA level in hypo-

thalamus of females was down-regulated by elevated concentration of circulating progesterone, as it is in the classic progesterone target tissues [31].

Our results indicated that in males the elevated concentration of circulating progesterone had no effects on neuropeptides or progesterone receptor mRNA level in hypothalamus. Too low serum concentration of progesterone could contribute to the lack of changes in gene expression. Despite the several-fold increase of circulating progesterone concentration in progesterone-treated males, the total concentration barely reached the value observed in non-treated control females (Table I). Consequently, the serum progesterone level in progesterone-treated males was approximately 2.5-fold lower than in progesterone-treated females.

No changes in the gene expression of neuropeptides in the hypothalamus of males suggest that despite the presence of progesterone receptor the serum concentration of this hormone may be a very important factor. It is worth noting that we likewise found no changes in gene expression in other organs of males treated with progesterone, unlike with females [4].

Conclusions

Our study demonstrates that chronic increase in circulating progesterone concentration in females is associated with up-regulation of NPY and down-regulation of CART genes expression in hypothalamus. These changes can lead to stimulation of appetite and could explain the observed increase in food intake, body, and adipose tissue mass in females. In males, elevated blood progesterone concentration had no effect on body and adipose tissue mass, and on the neuropeptide genes expression in hypothalamus. We also discovered that chronically elevated concentration of progesterone decreases PR gene expression in hypothalamus of females, similarly as observed in other tissues. It seems that the responsiveness of hypothalamus to progesterone may be dependent upon the serum concentration of this hormone.

Acknowledgments

We are indebted to Professor J. Swierczynski and Dr A. Hebanowska (Department of Biochemistry, Medical University of Gdansk) for criticism and discussion of the manuscript, and to Dr. Tomasz Sledzinski (Department of Pharmaceutical Biochemistry, Medical University of Gdansk) for his contribution to animals' operations.

References

1. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocr Rev 1997; 18: 502–519.
2. Grueso E, Rocha M, Puerta M. Plasma and cerebrospinal fluid leptin levels are maintained despite enhanced food intake in progesterone-treated rats. Eur J Endocrinol 2001; 144: 659–665.
3. Shirling D, Ashby JP, Baird JD. Effect of progesterone on lipid metabolism in the intact rat. J Endocrinol 1981; 90: 285–294.
4. Stelmanska E, Swierczynski J. Up-regulation of lipogenic enzyme genes expression in inguinal white adipose tissue of female rats by progesterone. J Steroid Biochem Mol Biol 2013; 134: 37–44.
5. Stelmanska E, Kmiec Z, Swierczynski J. The gender- and fat depot-specific regulation of leptin, resistin and adiponectin genes expression by progesterone in rat. J Steroid Biochem Mol Biol 2012; 132: 160–167.
6. Trujillo ML, Spuch C, Carro E et al. Hyperphagia and central mechanisms for leptin resistance during pregnancy. Endocrinology 2011; 152: 1355–1365.
7. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. Philos Trans R Soc Lond B Biol Sci 2006; 361: 1251–1263.
8. Wade GN. Some effects of ovarian hormones on food intake and body weight in female rats. J Comp Physiol Psychol 1975; 88: 183–193.
9. Pelkman CL, Chow M, Heinbach RA et al. Short-term effects of a progestational contraceptive drug on food intake, resting energy expenditure, and body weight in young women. Am J Clin Nutr 2001; 73: 19–26.
10. Lambert CP, Sullivan DH, Evans WJ. Megestrol acetate-induced weight gain does not negatively affect blood lipids in elderly men: effects of resistance training and testosterone replacement. J Gerontol A Biol Sci Med Sci 2003; 58: 644–647.
11. Oster MH, Enders SR, Samuels SJ et al. Megestrol acetate in patients with AIDS and cachexia. Ann Intern Med 1994; 121: 400–408.
12. Goodman RH, Regulation of neuropeptide gene expression. Annu Rev Neurosci 1990; 13: 111–127.
13. Jawiarczyk-Przybyłowska A, Bolanowski M. The role of orexin A in metabolic disturbances in patients with acromegaly. Endokrynol Pol 2012; 63: 463–469.
14. Sucajtys-Szulc E, Goyke E, Korczynska J et al. Refeeding after prolonged food restriction differentially affects hypothalamic and adipose tissue leptin gene expression. Neuropeptides 2009; 43: 321–325.
15. Sucajtys-Szulc E, Turyn J, Goyke E et al. Differential effect of prolonged food restriction and fasting on hypothalamic malonyl-CoA concentration and expression of orexigenic and anorexigenic neuropeptides genes in rats. Neuropeptides 2010; 44: 17–23.
16. Sucajtys-Szulc E, Goyke E, Korczynska J et al. Chronic food restriction differentially affects NPY mRNA level in neurons of the hypothalamus and in neurons that innervate liver. Neurosci Lett 2008; 433: 174–177.
17. Ducharme N, Banks WA, Morley JE et al. Brain distribution and behavioral effects of progesterone and pregnenolone after intranasal or intravenous administration. Eur J Pharmacol 2010; 641: 128–134.
18. Carrillo-Martinez GE, Gomora-Arrati P, Gonzalez-Arenas A et al. Role of progesterone receptors during postpartum estrus in rats. Horm Behav 2011; 59: 37–43.
19. O'Conner JL, Wade MF, Brann DW et al. Evidence that progesterone modulates anterior pituitary neuropeptide Y levels during the progesterone-induced gonadotropin surge in the estrogen-primed intact immature female rat. J Steroid Biochem Mol Biol 1995; 52: 497–504.
20. Brann DW, McDonald JK, Putnam CD et al. Regulation of hypothalamic gonadotropin-releasing hormone and neuropeptide Y concentrations by progesterone and corticosteroids in immature rats: correlation with luteinizing hormone and follicle-stimulating hormone release. Neuroendocrinology 1991; 54: 425–432.
21. Crowley WR, Tessel RE, O'Donohue TL et al. Effects of ovarian hormones on the concentrations of immunoreactive neuropeptide Y in discrete brain regions of the female rat: correlation with serum luteinizing hormone (LH) and median eminence LH-releasing hormone. Endocrinology 1985; 117: 1151–1155.
22. Turyn J, Stojek M, Swierczynski J. Up-regulation of stearoyl-CoA desaturase 1 and elongase 6 genes expression in rat lipogenic tissues by chronic food restriction and chronic food restriction/refeeding. Mol Cell Biochem 2010; 345: 181–188.
23. Ciosek J, Drobnik J. Galanin modulates oxytocin release from rat hypothalamo-neurohypophysial explant in vitro — the role of acute or prolonged osmotic stimulus. Endokrynol Pol 2013; 64: 139–148.
24. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156–159.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 2001; 25: 402–408.
26. Garcia MC, Lopez M, Gualillo O et al. Hypothalamic levels of NPY, MCH, and prepro-orexin mRNA during pregnancy and lactation in the rat: role of prolactin. FASEB J 2003; 17: 1392–1400.
27. Ladyman SR, Tups A, Augustine RA et al. Loss of hypothalamic response to leptin during pregnancy associated with development of melanocortin resistance. J Neuroendocrinol 2009; 21: 449–456.
28. Lima FB, Henderson JA, Reddy AP et al. Unique responses of midbrain CART neurons in macaques to ovarian steroids. Brain Res 2008; 1227: 76–88.
29. Kalra SP, Kalra PS. NPY and cohorts in regulating appetite, obesity and metabolic syndrome: beneficial effects of gene therapy. Neuropeptides 2004; 38: 201–211.
30. Tucholski K, Otto-Buczkowska E. The role of leptin in the regulation of carbohydrate metabolism. Endokrynol Pol 2011; 62: 258–262.
31. Katzenellenbogen BS. Dynamics of steroid hormone receptor action. Annu Rev Physiol 1980; 42: 17–35.