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Effect of testosterone supplementation on leptin release in rats after castration and/or unilateral surrenalectomy

Wpływ suplementacji testosteronu na uwalnianie leptyny u szczurów poddanych kastracji i/lub jednostronnej adrenalektomii

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Abstract

Introduction: The objective of this study was to examine the effect of testosterone supplementation on leptin release in rats which underwent castration and unilateral surrenalectomy.

Material and methods: The study was conducted on 80 adult male Wistar albino rats. Animals were divided into eight groups, with ten animals in each group. Group 1 was the Control group, Group 2 the Testosterone group, Group 3 the Castration group, Group 4 the Surrenalectomy group, Group 5 the Castration and Surrenalectomy group, Group 6 the Castration and Testosterone group, Group 7 the Surrenalectomy and Testosterone group, and Group 8 the Castration, Surrenalectomy and Testosterone group. The animals in Groups 2, 6, 7 and 8 were administered 5 mg/kg/day intramuscular testosterone propionate for four weeks. Blood samples were collected for analyses of leptin, LH, FSH and free and total testosterone levels in plasma.

Results: Groups 3 and 5 had the highest leptin and LH levels of all the groups ($p < 0.01$). Leptin and LH levels in Groups 1 and 4 were higher than those in Groups 2, 6, 7 and 8 ($p < 0.01$). A comparison of groups with regard to plasma FSH levels showed that the concerned parameter was significantly higher in Groups 3 and 5 than in the other groups ($p < 0.01$). FSH levels in Groups 1 and 4 were lower than those in all other groups ($p < 0.01$). The highest testosterone levels were obtained in Groups 2, 6, 7 and 8 ($p < 0.01$). Testosterone levels in Groups 1 and 4 were higher than those in Groups 3 and 5 ($p < 0.01$).

Conclusions: This study demonstrates that unilateral surrenalectomy in rats does not have a significant effect on leptin release, while plasma LH levels, rather than testosterone, may be more effective on plasma leptin. **(Pol J Endocrinol 2012; 63 (2): 119–124)**

Key words: *castration, surrenalectomy, testosterone supplementation, leptin*

Streszczenie

Wstęp: Celem badania była ocena wpływu suplementacji testosteronu na uwalnianie leptyny u szczurów poddanych kastracji i jednostronnej adrenalektomii.

Materiał i metody: Badanie przeprowadzono na 80 dorosłych samcach szczurów Wistar Albino. Zwierzęta podzielono na 8 grup, po 10 w każdej grupie, w następujący sposób: grupa 1. — grupa kontrolna, grupa 2. — suplementacja testosteronu, grupa 3. — kastracja, grupa 4. — adrenalektomia, grupa 5. — kastracja i adrenalektomia, grupa 6. — kastracja i suplementacja testosteronu, grupa 7. adrenalektomia i suplementacja testosteronu, grupa 8. — kastracja, adrenalektomia i suplementacja testosteronu. Zwierzętom z grup 2., 6., 7. i 8. podawano domięśniowo 5 mg/kg mc./d. propionianu testosteronu przez 4 tygodnie. Pobrano próbki krwi na oznaczenie stężeń leptyny, LH, FSH i wolnego oraz całkowitego testosteronu w osoczu.

Wyniki: U zwierząt z grup 3. i 5. stężenia leptyny i LH były wyższe niż w pozostałych grupach ($p < 0,01$). Stężenia leptyny i LH w grupach 1. i 4. były wyższe niż w grupach 2., 6., 7. i 8. ($p < 0,01$). Porównanie badanych grup pod względem stężeń FSH w osoczu wykazało, że parametr ten przyjmował istotnie większe wartości w grupach 3. i 5. niż w pozostałych grupach ($p < 0,01$). Stężenia FSH w grupach 1. i 4. były niższe niż w pozostałych ($p < 0,01$). Najwyższe stężenia testosteronu zaobserwowano w grupach 2., 6., 7. i 8. ($p < 0,01$). Stężenia testosteronu w grupach 1. i 4. były wyższe niż w grupach 3. i 5. ($p < 0,01$).

Wnioski: Wyniki badania wskazują, że jednostronna adrenalektomia u szczurów nie wpływa istotnie na wydzielanie leptyny. Sugerują również, że stężenie LH w osoczu silniej oddziałuje na osoczowe stężenie leptyny niż stężenie testosteronu. **(Endokrynol Pol 2012; 63 (2): 119–124)**

Słowa kluczowe: *kastracja, adrenalektomia, suplementacja testosteronu, leptyna*

Introduction

Leptin is the 167-amino-acid hormonal protein product of the obesity gene, and has been widely studied since 1994 [1]. Leptin, which was first defined in relation to satiety and energy balance, was later claimed to be an anti-obesity factor through its feedback effect from adipocytes to the hypothalamus. There has been increasing evidence demonstrating the importance of leptin in the regulation of body weight and food intake in both animals and humans [2]. Results of other studies indicate that leptin plays an important role in the

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regulation of metabolism [3], sexual development [4], reproduction [5], haematopoiesis [6], immunity [7], gastrointestinal functions [8], sympathetic activation [9] and angiogenesis [10].

It has been argued that leptin hormone, which is produced by adipocytes, has a fundamental role in regulating the body's energy balance, and also has significant effects on the reproductive system [11]. Preliminary studies in relation to the reproductive system, conducted on leptin-deficient ob/ob mice, show that such mice do not reach sexual maturity and are infertile, and that their levels of reproductive and gonadotropin hormones are low. However, the administration of leptin eliminates sterility. This situation is similar in humans [12]. The localisation of the leptin receptor in the hypothalamus-hypophysis axis suggests that leptin may have an important neuroendocrine role in the reproductive system [13]. Definition of leptin receptors in rat testes and Leydig cells may be a proof of a possible relation between this hormone and the male reproductive system [14].

It has been noted that leptin stimulates GnRH, LH and FSH release, forming strong signals that start puberty in both males and females [15]. However, leptin levels differ between the sexes. In males, leptin level increases in early childhood, reaching a peak in early puberty, then begins to decrease. Leptin levels in females have been found to be 3-4 times higher than those in males [16, 17]. Testosterone and testis volumes are in inverse proportion to leptin level after puberty [16]. It has been reported that exogenous testosterone supplementation to young males results in a significant decline in leptin levels [18], while it was argued in another study that leptin inhibited testosterone release in healthy males, but that testosterone was not a determining factor on the serum leptin level [19]. As a result, although some reports of previous studies are contradictory, it is now well known that there is a strong relation between leptin and the male reproductive system.

This study, which was conducted to find out the effects of unilateral surrenalectomy, castration and testosterone supplementation on leptin release in rats, may contribute to what is known in this field.

Materials and methods

Animal material and groups

This study was carried out in the laboratory of the Department of Physiology of Fırat University Medical Faculty, and included 80 adult male Wistar albino rats. The study protocol was approved by the ethics board of the said Faculty. All animals were weighed at the beginning and the end of the experiment.

The animals used in the study were equally allocated to eight groups:

— Group 1 (n = 10), Control Group; kept in the same conditions as the experimental groups, but not subjected to any procedure and fed on a normal diet;
— Group 2 (n = 10), Testosterone Group; fed on a normal diet and supplemented with 5 mg/kg/day intramuscular testosterone propionate for four weeks;
— Group 3 (n = 10), Castration Group; fed on a normal diet following a castration procedure performed under general anaesthesia;
— Group 4 (n = 10), Surrenalectomy Group; fed on a normal diet following a surrenalectomy procedure performed under general anaesthesia;
— Group 5 (n = 10), Castration and Surrenalectomy Group; fed on a normal diet following castration and surrenalectomy procedures performed under general anaesthesia;
— Group 6 (n = 10), Castration and Testosterone Group; supplemented with 5 mg/kg/day intramuscular testosterone propionate for four weeks and fed on a normal diet following a castration procedure performed under general anaesthesia;
— Group 7 (n = 10), Surrenalectomy and Testosterone Group; supplemented with 5 mg/kg/day intramuscular testosterone propionate for four weeks and fed on a normal diet following a surrenalectomy procedure performed under general anaesthesia;
— Group 8 (n = 10), Castration, Surrenalectomy and Testosterone Group; supplemented with 5 mg/kg/day intramuscular testosterone propionate for four weeks and fed on a normal diet following castration and surrenalectomy procedures performed under general anaesthesia.

The experimental animals were fed with standard rat pellet given daily at an amount of 10 g per 100 g of body weight. All injections were performed within 24 h following castration and surrenalectomy procedures between 9.00 and 10.00 a.m. After four weeks of testosterone injections, blood samples were collected for analysis from animals by decapitation between 9.00 and 10.00 a.m. Plasma samples were heparinised and kept at –80°C until the analysis time.

Animals in Groups 5 and 8 were anaesthetised and operated upon at the same time.

Procedures

Castration

Castration was surgically performed under general anaesthesia induced by a combination of rompun (5 mg/kg) and ketamine (60 mg/kg). After the scrotum was opened and funiculus spermaticus was ligated, the testes were removed. Scrotum skin was sutured,

and the sutures were removed on the 5th postoperative day [20].

Surrenalectomy

Surrenalectomy was surgically performed under general anaesthesia induced by a combination of rompun (5 mg/kg) and ketamine (60 mg/kg). Peritoneal cavity was accessed from the left side just above the last rib and the left surrenal gland was found. A small incision was made a few millimetres below the spinal muscles with intramuscular scissors. Blood vessels and tissues at the base of both ends of the forceps were clamped to remove the gland. The gland, which was first clamped and ligated, was then removed. After that, the incision was closed [20].

Testosterone supplementation

Testosterone propionate (testosterone propionate, T 1 875 Sigma Chemical Co. St Louis, MO, USA) supplementations were administered daily in the form of intramuscular injections containing 5 mg/kg testosterone propionate in 0.1 ml sesame oil.

Biochemical analyses

To determine the levels of plasma leptin, LH, FSH, free and total testosterone, 5 ml of blood samples were collected at the end of the study from all experimental animals by decapitation into heparinised tubes, centrifuged, and the plasma was separated then stored in plastic-capped tubes at –80°C until analysis.

Plasma leptin measurements

Plasma leptin was analysed using a Rat Leptin RIA (radioimmunoassay) test kit (Linco, catalogue no: RL-83K) with the help of a Gamma Counter (DPC Gambyt CR). Results were expressed as ng/mL.

Plasma LH measurements

LH in the plasma was analysed according to the RIA method using a Biocode rat LH kit (catalogue no: AH R002) using a Gamma Counter (DPC Gambyt CR). Results were presented as ng/mL.

Plasma FSH measurements

Plasma FSH analysis was carried out using a Biocode rat FSH kit (catalogue no: AH R004) according to the IRMA method (immunoradiometricassay) with the help of a Gamma Counter (DPC Gambyt CR). Results were expressed as ng/mL.

Plasma free testosterone measurements

Free testosterone measurements in plasma samples were made using a Coat-A-Count Testosterone Test Kit (catalogue no: TKTF1), with a Gamma Counter (DPC Gambyt CR) in line with the RIA method. Results were presented as pg/mL.

Plasma total testosterone measurements

Plasma total testosterone levels were determined using an Immulite commercial test (catalogue no: L2KTT2) in an Immulite 2000 autoanalyser according to the competitive immunoassay method. Results were expressed as ng/dL.

Statistical evaluations

Statistical evaluation of results was performed using a Minitab Windows Release 13.0 computer package software. Arithmetic means and standard deviations of all parameters were calculated. Variance analysis was employed to determine differences between groups. The level of significance was set at $p < 0.01$.

Results

An evaluation of the experimental animals showed that the mean body weights of all groups were not very different either before or after the study (Table I).

The highest plasma leptin and LH levels were obtained in Group 3 (castration) and Group 5 (castration and surrenalectomy), respectively ($p < 0.01$). Plasma leptin and LH levels in Group 1 (control) and Group 4 (surrenalectomy) were significantly higher than those in Group 2 (testosterone), Group 6 (castration and testosterone), Group 7 (surrenalectomy and testosterone) and Group 8 (castration, surrenalectomy and testosterone) ($p < 0.01$). Groups 2, 6, 7 and 8 were not different with regard to plasma leptin and LH values (Table II).

When plasma FSH levels of all groups were compared, FSH levels in Group 3 (castration) and Group 5 (castration and surrenalectomy) were significantly higher than those in all other groups ($p < 0.01$). The lowest plasma FSH levels were observed in Group 1 (control) and Group 4 (surrenalectomy) ($p < 0.01$). No significant difference was found between plasma FSH levels of Group 2 (testosterone), Group 6 (castration and testosterone), Group 7 (surrenalectomy and testosterone) and Group 8 (castration, surrenalectomy and testosterone) (Table II).

The highest plasma free and total testosterone levels were obtained in Group 2 (testosterone), Group 6 (castration and testosterone), Group 7 (surrenalectomy and testosterone) and Group 8 (castration, surrenalectomy and testosterone) ($p < 0.01$). The lowest plasma free and total testosterone levels were found in Group 3 (castration) and Group 5 (castration and surrenalectomy) ($p < 0.01$). Plasma free and total testosterone levels in Group 1 (control) and Group 4 (surrenalectomy) did not show any differences (Table III).

Table I. *Mean weights of animals before and after the study*
Tabela I. *Średnia masa ciała zwierząt przed i po badaniu*

Groups (n = 10)	Before experiments [g]	After experiments [g]
1 Controls	150.25 ± 11.20	155.30 ± 12.40
2 Testosterone	152.05 ± 10.45	155.50 ± 12.75
3 Castration	150.65 ± 12.50	156.70 ± 11.80
4 Surrenalectomy	153.00 ± 09.90	158.60 ± 10.90
5 Castration and Surrenalectomy	151.15 ± 10.10	157.20 ± 11.40
6 Castration and Testosterone	152.75 ± 10.40	158.35 ± 12.20
7 Surrenalectomy and Testosterone	152.40 ± 11.25	158.24 ± 13.70
8 Castration, Surrenalectomy and Testosterone	153.95 ± 10.40	159.70 ± 13.50

Table II. *Plasma leptin, LH and FSH levels in the study groups**
Tabela II. *Stężenia leptyny, LH i FSH w osoczu w poszczególnych grupach*

Groups (n = 10)	Leptin [ng/mL]	LH [ng/mL]	FSH [ng/mL]
1 Controls	3.15 ± 0.25^{B}	5.75 ± 0.70^{B}	9.80 ± 1.20^{C}
2 Testosterone	1.45 ± 0.50^{C}	2.95 ± 0.20^{C}	21.60 ± 3.15^{B}
3 Castration	11.10 ± 1.70^{A}	13.55 ± 1.85^{A}	90.12 ± 8.70^{A}
4 Surrenalectomy	3.25 ± 0.18^{B}	5.20 ± 0.90^{B}	10.30 ± 1.50^{C}
5 Castration and Surrenalectomy	10.95 ± 1.40^{A}	14.00 ± 2.00^{A}	91.75 ± 9.15^{A}
6 Castration and Testosterone	1.70 ± 0.40^{C}	3.20 ± 0.40^{C}	19.75 ± 2.70^{B}
7 Surrenalectomy and Testosterone	1.55 ± 0.30^{C}	3.10 ± 0.45^{C}	22.00 ± 2.08^{B}
8 Castration, Surrenalectomy and Testosterone	1.60 ± 0.25^{C}	3.00 ± 0.50^{C}	21.90 ± 3.10^{B}
p	0.01	0.01	0.01

*Differences between means with different superscripted letters in the same column are statistically significant ($p < 0.01$); A > B > C

Table III. *Plasma free and total testosterone levels in the study groups**
Tabela III. *Stężenie wolnego i całkowitego testosteronu w osoczu w poszczególnych grupach*

Groups (n = 10)	Free Testosterone [pg/mL]	Total Testosterone [ng/dL]
1 Controls	5.20 ± 0.75^{B}	195.80 ± 18.40^{B}
2 Testosterone	16.05 ± 3.20^{A}	310.60 ± 20.40^{A}
3 Castration	0.02 ± 0.00^{C}	20.00 ± 0.00^{C}
4 Surrenalectomy	4.95 ± 0.80^{B}	192.25 ± 17.50^{B}
5 Castration and Surrenalectomy	0.02 ± 0.00^{C}	20.00 ± 0.00^{C}
6 Castration and Testosterone	14.80 ± 2.20^{A}	308.56 ± 21.30^{A}
7 Surrenalectomy and Testosterone	15.25 ± 2.15^{A}	311.56 ± 21.30^{A}
8 Castration, Surrenalectomy and Testosterone	14.75 ± 1.75^{A}	306.20 ± 19.60^{A}
p	0.01	0.01

*Differences between means with different superscripted letters in the same column are statistically significant ($p < 0.01$); A > B > C

Discussion

Mean body weights of all groups were not different either at the beginning or at the end of the study. Leptin is a recently defined protein-structure hormone synthesised and released by adipose tissue [21, 22]. The idea that adipose tissue might control body weight by secreting a hormone was first put forward in 1953 when it was called the lipostatic theory [23]. Leptin level is in a positive relation with fatty tissue mass. Obese people have a higher leptin mRNA and higher plasma leptin levels. Leptin levels rapidly decrease during periods of fasting [24]. The fact that no significant difference emerged in the body weights of all groups throughout the study might enable a better discussion of the relation between the procedures conducted in the experiments and leptin release.

Our study obtained the highest leptin levels in Group 3 (castration) and Group 5 (castration and surrenalectomy) where we induced testosterone deficiency by a castration procedure. The results of studies examining the relation between leptin and testosterone are inconsistent [16, 18]. A study including male rats has indicated that the leptin level increases parallel to an increase in testosterone level, thus making the inhibitory role of testosterone on leptin questionable [25]. Ahima et al. [26] reported that leptin treatment in male rats with food deprivation significantly reduced the decline in LH and testosterone. However, other rat studies demonstrated that leptin did not influence testicular steroidogenesis [27, 28]. A study including old hypogonadal males reported that an increased level of leptin in circulation was not associated with advanced age or decreased testosterone [29]. Similarly, Bray and York [30] claimed that there was no significant correlation between serum testosterone and leptin.

Our study demonstrates that 4-week testosterone deficiency leads to a significant increase in plasma leptin levels. In other words, a decreased testosterone level results in an increased leptin level. In a study including hypogonadal patients without any operation, Behre et al. [31] found low serum testosterone together with increased leptin levels. In the same study, exogenous androgen was supplemented to some of the hypogonadal patients, and increased testosterone and decreased leptin levels were obtained thereafter. This study concluded that there was a negative correlation between serum leptin and testosterone. A similar result was reported by Luukkaa et al. [18], who established a negative correlation between testosterone and leptin in 269 non-diabetic males. They demonstrated that 12-month exogenous testosterone supplementation to ten voluntary healthy males significantly reduced leptin levels, and determined that when testosterone treatment was interrupted in this group, leptin concentration was restored to pre-treatment levels. In that respect, the high leptin levels we found in Group 3 (castration) and Group 5 (castration and surrenalectomy) in which we induced testosterone deficiency by castration are consistent with the findings of Behre et al. [31] and Luukkaa et al. [18].

In our study, we also established an inverse relation between testosterone and leptin in Groups 2 (testosterone), 6 (castration and testosterone), 7 (surrenalectomy and testosterone) and 8 (castration, surrenalectomy and testosterone), to which we supplemented testosterone. The highest free and total testosterone levels were obtained in these groups, which also had the lowest plasma leptin levels. Our observation of decreased leptin and increased free and total testosterone levels in Groups 2, 6, 7 and 8 reveals the inverse correlation between testosterone and leptin from another perspective. However, we noted that in some studies examining the relation between testosterone and leptin [18, 31], there were no analyses of LH and FSH. In our study, we wanted to examine not only the leptin-testosterone relation, but also the correlation between this hormone and LH and FSH.

Group 3 (castration) and Group 5 (castration and surrenalectomy), where we obtained the highest leptin levels, also had the highest plasma LH and FSH levels. Studies investigating the relation between leptin and LH and FSH have tended to focus more on the way this hormone affects LH and FSH. It is an accepted fact that leptin is influenced by steroid hormones and can be an important signal in the development of puberty through its stimulation of LH release [32]. It has been reported that leptin directly stimulates LH release, and to a lesser extent FSH release through NO activation in gonadotropes [15]. It was reported in a study examining the effects of leptin on gonadotropin secretion in fasted male apes that leptin prevented the inhibition of plasma LH and FSH levels caused by hunger [33]. The high LH and FSH levels we obtained in Group 3 and Group 5 constitute an expected result that should be found due to testosterone deficiency following castration.

However, the point that needs to be addressed here is whether the main factor influencing leptin release is testosterone or LH and FSH. We hope to answer this question in the following parts where we discuss findings pertinent to other groups. However, it can be safely stated that the correlation between leptin and LH and FSH does not appear to be unidirectional. If leptin increases LH and FSH levels, it can be speculated that LH and FSH have an effect on plasma leptin. The fact that the increase in LH and FSH arising from testosterone deficiency in rats in our study was accompanied by an increase in leptin concentration indicates that

this relation is bidirectional. Decreased levels of LH and FSH parallel to decreased leptin levels in Group 2 (testosterone), Group 6 (castration and testosterone), Group 7 (surrenalectomy and testosterone) and Group 8 (castration, surrenalectomy and testosterone) compared to groups in which testosterone was not supplemented and to those groups that were castrated (groups 3 and 5) to which we supplemented testosterone, also support our view that the correlation between leptin and LH and FSH is not unidirectional.

In our study, plasma leptin, LH, FSH as well as free and total testosterone levels in Group 4 where we performed only surrenalectomy were not different from Group 1, which was not subjected to any procedure. The idea of performing surrenalectomy arose from the aim of revealing whether androgens secreted from the adrenal gland cortex affect plasma leptin or not. We found no study on this topic in the literature. Another important result of the study we carried out is that unilateral surrenalectomy does not have a significant effect on leptin release.

Conclusion

This is the first study where testosterone supplementation, castration and surrenalectomy were performed both individually and in combination simultaneously in rats.

We drew four conclusions from our study:

1. Castration procedure significantly increases plasma leptin level in rats.
2. Unilateral surrenalectomy does not have a significant effect on leptin release in rats.
3. Testosterone supplementation results in a reduced leptin level.
4. Increase in leptin levels caused by castration may be inhibited by testosterone supplementation.

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