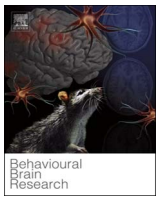
Behavioural Brain Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Effects of chronic testosterone administration on the degree of preference for a high-fat diet and body weight in gonadal-intact and ovariectomized female rats

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ARTICLE INFO

Keywords:
Testosterone
Body weight
High-fat diet preference
Ovariectomy

ABSTRACT

Energy balance and reproductive functions are closely linked in some species. The sex hormones (estrogens and androgens) are involved in the regulation of appetite, metabolism, body weight (BW), and body composition in mammals. Previously, we showed that the effects of testosterone on BW, appetite, and fat weight were markedly affected by alterations to the gonadal hormonal milieu. In this study, we examined whether testosterone administration changes food preferences and whether these effects of testosterone depend on gonadal status in female rats. We also evaluated the underlying mechanisms responsible for these effects, focusing on hypothalamic inflammation and endoplasmic reticulum (ER) stress. In gonadal-intact (sham) female rats, chronic testosterone administration promoted a preference for a high-fat diet (HFD) and increased BW gain, fat weight, and adipocyte size, whereas no such effects were observed in ovariectomized (OVX) rats. Testosterone administration increased hypothalamic interleukin-1 mRNA expression in the sham rats, but not the OVX rats. On the contrary, testosterone administration decreased the hypothalamic mRNA levels of ER stress-response genes in the OVX rats, but not the sham rats. These testosterone-induced alterations in OVX rats might represent a regulatory mechanism for preventing hypothalamic inflammation and the overconsumption of a HFD. In conclusion, testosterone's effects on food preferences and the subsequent changes were affected by gonadal status. Testosterone-induced changes in hypothalamic inflammatory cytokine production and ER stress might be related to these findings.

1. Introduction

It has been reported that energy balance and reproductive functions are closely linked in some species. The sex hormones (estrogens and androgens) contribute to the regulation of appetite, metabolism, body weight (BW), and body composition in mammals [1]. In females, estrogens play roles in preventing excessive BW gain and obesity. Food intake and BW are increased in ovariectomized (OVX) female animals, and these effects of ovariectomy can be counteracted via estradiol replacement [1–5]. On the contrary, the roles of androgens in BW regulation, appetite, and metabolism in females have not been fully clarified, although some studies have indicated that androgens might induce appetite dysregulation [1,6–8] and visceral adiposity in females and experimental animals [1,9–13]. Most of these previous studies focused on the effects of androgens in humans and experimental animals of reproductive age, whereas there are only limited data on the roles of androgens in the regulation of appetite and BW at other life stages, such as the menopause. In our previous studies, we showed that the effects of testosterone on BW, appetite, and fat weight were markedly affected by alterations in the gonadal hormonal milieu [14,15]. The chronic administration of testosterone increased BW, appetite, and fat weight in gonadal-intact and OVX plus estradiol-supplemented rats, whereas testosterone caused reductions in these factors in OVX rats. These findings indicate that the effects of androgens on the regulation of BW, appetite, and metabolism might depend on the estrogenic milieu and reproductive age.

As noted above, the effects of androgens on BW and appetite are markedly affected by the estrogenic milieu [14,15]. However, because the aforementioned studies were performed under fixed feeding conditions; i.e., standardized food was supplied to all experimental animals, the effects of androgens on food preferences could not be evaluated. As humans encounter various feeding environments, and most

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https://doi.org/10.1016/j.bbr.2018.02.021
Received 4 January 2018; Received in revised form 27 January 2018; Accepted 18 February 2018

humans can freely select their favorite foods, the effects of androgens on dietary habits have significant implications.

In the present study, we examined whether the administration of testosterone affects food preferences and whether these effects depend on gonadal status in female rats. The hypothalamic levels of various factors involved in appetite and inflammation were measured to evaluate the mechanisms that underlie the effects of testosterone. We also examined the effects of testosterone on the expression of hypothalamic endoplasmic reticulum (ER) stress-response genes and leptin because these factors promote a preference for a high-fat diet (HFD) [16–18].

2. Materials and methods

2.1. Animals

Adult female Sprague-Dawley rats were purchased and housed under controlled lighting (14 h light, 10 h dark cycle) and temperature (24 ℃) conditions. All animal experiments were conducted in accordance with the ethical standards of the animal care and use committee of the University of Tokushima.

2.2. Chronic testosterone administration in sham-operated and ovariectomized female rats

Adult female rats were randomly divided into sham-operated (sham) and bilaterally ovariectomized (OVX) groups. All surgical procedures were carried out under sodium pentobarbital-induced anesthesia. In the sham groups, the ovaries were just touched with forceps. Four-six weeks after the operation, the rats of each group were further divided into control and testosterone-administered groups. Each rat in the testosterone-administered group was implanted with a silastic tube filled with crystalline testosterone (internal diameter: 3 mm; length of filled part: 10 mm in the sham group and 15 mm in the OVX group), and each rat in the control group was implanted with an empty tube. After surgery, the rats were housed individually, and BW, calorie intake, and the extent of the rats' preference for fat was measured weekly after the implantation procedure. Feed efficiency was calculated as follows: BW gain/energy intake.

2.3. High-fat diet preference

The rats' preference for a HFD was evaluated using standard-chow (SC) (type MF; Oriental Yeast Co. Ltd., Tokyo, Japan; 359 kcal/100 g, 12.8% of the provided calories were derived from fat) and a HFD (HFD-60; Oriental Yeast Co. Ltd., Tokyo, Japan; 506.2 kcal/100 g, 62.2% of the provided calories were derived from lard-based fat). The intake of the SC and HFD was measured and analyzed to assess the rats' preference for fat. HFD preference was calculated as follows: (energy intake from HFD/total energy intake) ×100.

2.4. Tissue sampling

At 3 weeks after the implantation procedure, the rats were killed by decapitation under sevoflurane-induced anesthesia, and the weights of visceral fat, subcutaneous fat, and the uterus were measured. The brain, visceral and subcutaneous fat, and blood were collected. Serum was separated by centrifugation and stored at −20 ℃, and the brain and fat samples were stored at −80 ℃.

2.5. Hormone assay

Serum leptin levels were measured using a 125I-radioimmunoassay (RIA) kit (multi-species RIA kit, Linco Research Inc., MO, USA). The sensitivity of the assay was 1.0 ng/ml, and its inter- and intra-assay coefficients of variation were 3.2% and 7.8%, respectively.

2.6. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously [14,15]. Briefly, the brain sections were dissected out via an anterior coronal cut 2 mm anterior from the optic chasm, a posterior coronal cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total RNA was isolated from the hypothalamic explants and visceral fat using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen Gmbh, Hilden, Germany). cDNA was synthesized with oligo (deoxythymidine) primers at 50 ℃ using the SuperScript III first-strand synthesis system for the real-time polymerase chain reaction (PCR; Invitrogen Co.). The PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. Standard curves generated from 4-fold dilution series of a sample were used for the relative quantification of the expression levels of each factor. The expression levels were normalized by dividing them by the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Dissociation curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. The primer sequences and annealing temperatures are shown in Table 1. The PCR conditions were as follows: the initial denaturation and enzyme activation were carried out at 95 ℃ for 20 s, followed by 45 cycles of denaturation at 95℃ for 3 s, and annealing and extension for 30 s.

2.7. Histology

Fixed visceral fat was dehydrated with ethanol and xylene and sliced into sections after being embedded in paraffin. Serial 4-μm-thick sections were stained with hematoxylin and eosin (H&E), and histological images were captured by a Zeiss Imager M2 microscope using the AxioVision version 4.8 acquisition software (Zeiss). The mean adipocyte area was determined using the software ImageJ.

2.8. Statistical analyses

All results are presented as mean ± standard error of the mean (SEM) values. Two-way repeated analysis of variance (ANOVA) and the Student's *t*-test were used for comparisons between the control and testosterone-administered groups. Student's *t*-test was also used for the comparisons between the sham-control and OVX-control groups. P-values of < 0.05 were considered significant.

Table 1
Primer sequences, product sizes and annealing temperature.

Primer	Sequence	Annealing T (℃)
OBRb forward	GCA GCT ATG GTC TCA CTT CTT TTG	63
OBRb reverse	GGT TCC CTG GGT GCT CTG A	
NPY forward	GGG GCT GTG TGG ACT GAC CCT	66
NPY reverse	GAT GTA GTG TCG CAG AGC GGA G	
IL-1 forward	GCT GTG GCA GCT ACC TAT GTC TTG	66
IL-1 reverse	AGG TCG TCA TCA TCC CAC GAG	
TNF-α forward	AGC CCT GGT ATG AGC CCA TGT A	65
TNF-α reverse	CCG GAC TCC GTG ATG TCT AAG T	
Xbp1S forward	GAG TCC GCA GCA GGT G	61
Xbp1S reverse	GCG TCA GAA TCC ATG GGA	
Xbp1U forward	GCC CTG GTT ACT GAA GAG GT	58
Xbp1U reverse	CAC GTA GTC TGA GTG CTG C	
CHOP forward	CAC AAG CAC CTC CCA AAG	59
CHOP reverse	CCT GCT CCT TCT CCT TCA T	
GAPDH forward	ATG GCA CAG TCA AGG CTG AGA	64
GAPDH reverse	CGC TCC TGG AAG ATG GTG AT	