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Effect of chronic administration of tamoxifen and/or estradiol on feeding behavior, palatable food and metabolic parameters in ovariectomized rats

Carine Lampert, Danusa Mar Arcego, Daniela P. Laureano, Luísa A. Diehl, Isadora Ferreira da Costa Lima, Rachel Krolow, Letícia F. Pettenuzzo, Carla Dalmaz, Deusa Vendite *

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde — ICBS, UFRGS (Saúde), RS, Brazil

HIGHLIGHTS

- Tamoxifen treatment mimicked the effects of estradiol in many parameters analyzed.
- Under restrictive schedule, TAM treated rats ate as much Froot Loops as E group.
- All groups prefer chocolate than standard chow, but E group ate even more chocolate.
- TAM group showed lower caloric efficiency irrespective of the kind of food offered.
- Treatment with estradiol and tamoxifen showed a favorable lipid profile.

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ABSTRACT

Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM) used in the treatment of breast cancer; however many women complain of weight gain during TAM treatment. The anorectic effects of estradiol (E) and TAM are well known, although the effects of E on the consumption of palatable food are controversial and there is no information regarding the effects of TAM on palatable food consumption. The aim of this study was to investigate the effects of chronic treatment with estradiol and/or tamoxifen on feeding behavior in ovariectomized rats exposed to standard chow and palatable foods (Froot Loops® or chocolate). Additionally, parameters such as body weight, uterine weight, lipid profile and plasma glucose were also measured. Wistar rats were ovariectomized (OVX) and subsequently injected (ip.) for 40 days with: E, TAM, E + TAM or vehicle (OVX and SHAM – controls). Behavioral tests were initiated 25 days after the start of treatment. Froot Loops® consumption was evaluated in a novel environment for 3 min. Standard chow intake was evaluated for two days and chocolate intake for 7 days in the home cage in a free choice model (chocolate or standard chow). Rats injected with E, TAM and E + TAM groups showed a reduction in body weight and standard chow intake, compared with control groups. With regard to palatable food intake, the E, TAM and E + TAM groups demonstrated increased consumption of Froot Loops®, compared with the SHAM and OVX groups. In contrast, all groups increased their consumption of chocolate, compared with standard chow; however the E group consumed more chocolate than the OVX, TAM and E + TAM groups. Despite these differences in chocolate consumption, all groups showed the same caloric intake during the chocolate exposure period; however the TAM and E + TAM groups presented decreased body weight. Treatment with estradiol and tamoxifen showed a favorable lipid profile with low levels of TC, LDL, LDL/HDL ratio and lower levels of plasma glucose. The E group presented high levels of TG and HDL, when compared with the TAM and E + TAM groups. Taken together, results suggest that TAM acted in an estrogen-like manner on the majority of parameters analyzed. However, tamoxifen acts in a different manner depending on the type of palatable food and the exposure. In addition, the TAM group demonstrated weight loss, compared with other groups independently of the type of food presented (palatable food or standard chow), showing a low caloric efficiency.

1. Introduction

Tamoxifen (TAM) is a triphenethylene derivative drug that is widely used for the treatment of all stages of breast cancer [1], reducing the incidence of breast cancer in both pre and postmenopausal

* Corresponding author at: Departamento de Bioquímica, ICBS, UFRGS, Ramiro Barcelos, 2600 (Anexo) Lab 37, CEP: 90035-003, Porto Alegre, RS, Brazil. Tel.: +55 51 3308 5570; fax: +55 51 3308 5535.
E-mail address: vendite.ez@terra.com.br (D. Vendite).

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women at elevated risk [2,3]. TAM is a selective estrogen receptor modulator (SERM), a class of drugs that act as estrogen receptor agonists or antagonists, depending on the target tissue [4]. TAM behaves like an estrogen antagonist in mammary tissue while it mimics the effects of estrogen in other tissues, for example the uterus, cardiac and bone tissues [5–7]. However, it is not yet understood whether TAM has estrogenic or antiestrogenic activities in the brain.

Estrogens exert their physiological effects through two estrogen receptor (ER) subtypes, ERα and ERβ, which belong to the nuclear receptor family of ligand-activated transcription factors. ER has two 'activation' domains within the receptor, which facilitate the interaction of the ER with the transcription apparatus-activation function-1 (AF-1) and activation function-2 (AF-2). Both ERα and ERβ contain an AF-2 domain, but unlike ERα, ERβ seems to have a weaker AF-1 domain and depends more on the AF-2 for its transcriptional activation function [8,9]. Furthermore, estrogen can act by non-genomic mechanisms via membrane ERs [10]. Evidence suggests that tamoxifen inhibits ER-AF-2 activity, and consequently acts as an antagonist in ERβ and has partial agonist activity in ERα [11].

In addition to the estrogen effects on reproductive physiology, this ovarian hormone can modulate numerous brain neurotransmitters and neuromodulators, including the serotonergic, dopaminergic, neuropeptide Y and opioidergic systems, and consequently modulate cognitive functions, mood and the reward system [12–14]. It is well established that estradiol reduces food intake, body weight and improves lipid profile, possibly via the activation of ERα [15,16]. These effects are probably due to an increase in the expression of anorexigenic genes and a decrease in the expression of orexigenic genes [17]. It is well known that tamoxifen mimics the effects of estradiol on food intake in rats [18,19].

Apart from these well-established effects of estradiol on food intake, the role of estradiol on palatable food is controversial and more complex [20,21]. Palatable foods activate the reward system, thereby affecting ingestive behavior [22,23]. The predilection for palatable foods and reward system activation is a basic and evolutionarily-conserved survival mechanism in animals and humans [24]. Foods rich in fat and sugar are attractive because such foods can be rapidly converted into energy [24,25]. It has been demonstrated that the preference for palatable foods, including sweets, differs between males and females, and sweet preferences change across the menstrual cycle and during pregnancy [20,26–30]. However, other studies report that estrogen does not affect behavioral responses to palatable foods [21,31].

Although it is well established that tamoxifen-treated animals demonstrate reduced body weight and standard chow intake, there is no information in the literature regarding tamoxifen treatment and the consumption of palatable food. On the other hand, humans have access to a variety of fat and sweet foods and it is known that many women experience weight gain during tamoxifen treatment for breast cancer. This is especially true for patients who were not overweight before diagnosis [19,32].

Therefore, the aim of this study was to investigate the effects of chronic treatment with estradiol and/or tamoxifen on feeding behavior in ovariectomized rats exposed to standard chow and palatable foods (Froot Loops® or chocolate). Additionally, parameters such as body weight, uterine weight and lipid profile were also measured.

2. Methods

2.1. Animals

We used 120 adult female (7–13 per group), Wistar rats (75 days of age at the beginning of the treatment), weighing between 180 and 220 g. Rats were housed in groups, with five rats per cage. Cages were made of Plexiglas material (65 × 25 × 15 cm) with the floor covered with sawdust. Animals were maintained on a standard dark–light cycle (lights on between 7:00 h and 19:00 h), at a room temperature of 21 ± 1 °C. The rats had free access to food (standard rat chow) and water. All experimental procedures occurred during the light phase (10:00–15:00 h). All animal treatments were in accordance with the institutional guidelines and according to the recommendations of the International Council for Laboratory Animal Science (ICLAS), and all efforts were made to minimize animal suffering, as well as to reduce the number of animals used.

2.2. Surgery

All rats were ovariectomized or just underwent the surgery without removal of ovaries (SHAM group). Ovariectomies were performed under aseptic conditions. Rats were anesthetized with 60 mg/kg ketamine HCl (Dopalen: Agribrands, Campinas, SP, Brazil) and 16 mg/kg xylazine (Anasedan: Agribrands, Campinas, SP, Brazil) ip., and bilateral ovariectomy was performed with a single abdominal incision. The abdominal skin was then cut, the peritoneum was opened, both ovarian arteries were linked, and both ovaries were removed. The muscle and the skin were sutured [33]. Animals received one drop of acetaminophen (200 mg/ml, Paracetamol EMS, Hortolândia, SP, Brazil) after surgery as analgesic.

2.3. Treatments

After a recovery period of 10–15 days, the animals received Froot Loops® or chocolate in their home cage, 24 h before beginning the treatments in order to avoid taste aversion [34,35]. The animals were divided into five groups: SHAM group (submitted to the surgery without removal of ovaries and received vehicle), OVX group (received vehicle), 17β-estradiol group (OVX that received 0.1 mg/kg), tamoxifen group (OVX that received 2 mg/kg) and E + TAM (OVX that received 0.1 mg/kg of 17β-estradiol + 2 mg/kg of tamoxifen). Tamoxifen (PharmaPlus — Porto Alegre, RS, Brazil) and estradiol (Sigma — St. Louis, MO, USA) was dissolved in vehicle: ethanol (10%), DMSO (5%) and 0.9% NaCl (85%). It is important to note that the 2 mg/kg dose of tamoxifen used in the present study is calculated to be the equivalent dose that is prescribed to patients (20 mg), based on surface area (mg/m^2) [34]. The 0.1 mg/kg dose of estradiol was chosen based on previous studies [36], and mimics the proestrus phase of the estrous cycle [37]. Rats were injected (1 ml/kg ip.) daily between 1:00 pm and 3:00 pm until euthanasia. Body weight was monitored once a week.

2.4. Exposure to sweet food

The behavioral test was initiated 25 days after the first injection. Prior to all tests, rats were acclimatized to the experimental room for at least 30 min. This food behavior was conducted during the light phase [28,38], in order to measure the consumption of sweet food regardless of physiological mechanisms of hunger.

Rats were placed in a rectangular box (40 cm × 15 cm × 20 cm) with floor and side walls made of wood and a glass ceiling. Ten Froot Loops® (Kellogg's® pellets of wheat, cornstarch and sucrose) were placed in one extremity of the box. The animals were habituated to this environment for 5 days, for 3 min each day, under food restriction (receiving 80% of habitual ingestion). After the last habituation session, the animals were fed ad libitum and were exposed to a 3-min test session, 24 h later. Time spent to reach the food, time spent until beginning to eat and the number of ingested Froot Loops® were evaluated in each trial and in the test session. A protocol was established so that when the animals ate part of the Froot Loops® (e.g., 1/3 or 1/4), this fraction was considered [38]. This experiment could mimic the problem of women that gain weight during TAM treatment and diet in order to lose weight using food restriction.