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Effects of selective estrogen receptor agonists on food intake and body weight gain in rats

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Abstract

Ovariectomized (OVX) rats eat more and gain weight more rapidly than sham-operated (SO) rats and estradiol (E_2) treatment attenuates food intake and body weight gain in OVX rats. Studies were designed to test the hypothesis that the alpha subtype of the estrogen receptor (ERα) mediates the attenuating effects of E_2 on food intake and body weight gain while the beta subtype (ERβ) mediates opposing actions that lead to increased food intake and body weight gain. Female rats were SO or OVX and treated daily with vehicle (dimethylsulfoxide, DMSO) or E_2 (10 μg/day), or the ERα−selective agonist, 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT, 0.5 mg/day), or the ERβ−selective agonist, 2,3-bis(4-hyroxyphenyl)-propionitrile (DPN, 0.5 mg/day) for 14 days. Total food intake was significantly reduced by E_2 and PPT, but not DPN. Total body weight gain was significantly increased in OVX rats compared to SO rats and treatment with E_2 or PPT, but not DPN, significantly decreased total body weight gain to levels that were not significantly different from SO rats. A dose−response study of PPT indicated that at 0.25 mg/day, PPT significantly reduced total 21-day food intake and body weight gain and, at 0.13 and 0.06 mg/day, PPT significantly reduced total body weight gain compared to OVX rats without significantly reducing total food intake. A dose−response study of DPN indicated that none of the three doses of DPN significantly altered total 21-day food intake or total body weight gain. These results suggest ERα mediates the attenuating effects of estrogens on food intake and body weight gain while ERβ has no effect on these variables.

Keywords: Ovariectomized rats; Sham-operated; Food intake; Body weight gain

1. Introduction

In the peri-menopausal years women gain an average of about 2 kg [1] and clinical data indicate that estrogens are important modulators of body weight. For example, the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial followed women given placebo or one of a number of hormone replacement therapy (HRT) regimens, for 3 years. At the end of the study, weight gain was greatest in the placebo group compared to any of the HRT groups and the least weight gain was seen in the group treated with unopposed conjugated equine estrogens [2]. Data such as these suggest that estrogen-deficiency during menopause contributes to an increase in body weight gain and raises the possibility of developing pharmaceuticals to prevent postmenopausal weight gain.

Inhibitory effects of estrogens on body weight gain have been recognized in animal models for a number of years [3]. Ovariectomized (OVX) rats are hyperphagic and gain more weight than sham-operated control rats [4–7]. It has been suggested that the effects of ovariectomy and estrogens on body weight gain are mediated by an inhibitory effect of estrogen on food intake [8]. However, several studies have found that OVX-induced weight gain and estrogen attenuation of OVX-induced weight gain are not completely abolished by pair feeding [9–11], suggesting that estrogens decrease body weight via dual effects on food intake and metabolic expenditure.

Current evidence suggests that ERα and ERβ may differentially regulate body weight gain. Adult ERα knockout mice show an increase in white adipose tissue accumulation in various regions compared to wild-type (WT) mice [12] and E_2 does not attenuate body weight gain or enhance cholecystokinin (CCK)-induced signaling in ERα knockout mice [13], suggesting ERα mediates the attenuating effects of estrogens on body weight

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gain. In contrast, the role of ERβ in mediating the effects of estrogens on body weight gain is more controversial. One study showed that in 12- to 13-week-old OVX ERα knockout mice, treatment with E_2 increases weight gain and fat accumulation more than in untreated OVX ERα knockout mice [14], suggesting a role for ERβ in adipose tissue accumulation. In contrast, a study of 9-week-old OVX ERα knockout mice showed no effect of E_2 on body weight gain compared to OVX ERα knockout mice treated with vehicle, suggesting no role for ERβ in mediating body weight [13]. A study of male ERα knockout, ERβ knockout, and ERα/ERβ double knockout mice has been interpreted as suggesting age is the reason for these apparent conflicting results [15]. Total body fat, as measured by dual X-ray absorptiometry, was increased in ERα knockout and ERα/ERβ double knockout mice and in ERβ knockout mice retroperitoneal fat pad weights tended to be decreased as compared to WT [15]. This increase in adipose accumulation in the ERα knockout and ERα/ERβ double knockout mice and tendency towards a decrease in adipose tissue accumulation in ERβ knockout mice was observed in adult 16-week old but not 4-week-old prepubertal or 8-week-old pubertal mice, suggesting to these authors that the inhibitory effect of ERα and the stimulatory effects of ERβ on body weight gain do not become readily apparent in the knockout mice until the animals reach adulthood [15]. However, this interpretation of the data should be accepted cautiously as the tendency towards a decrease in the retroperitoneal fat pad in the ERβ knockout mice was not statistically significant [15]. Therefore, the knockout data in this study more accurately supports no role for ERβ in body weight maintenance.

Another study found that the inhibitory effect of E_2 on food intake and body weight gain was blocked by intracerebroventricular treatment with anti-sense oligodeoxynucleotides directed against ERβ and intracerebroventricular administration of anti-sense oligodeoxynucleotides directed against ERα had no effect. This study suggests that in the central nervous system ERβ mediates the inhibitory effects of estrogens on body weight gain [16] and raises the possibility that the roles of peripheral and central ERα and ERβ in body weight maintenance differ. Together, these reports leave the roles of ERα and ERβ in mediating the effects of estrogens on food intake and body weight gain unclear.

The purpose of this study was to use two selective estrogen receptor agonists that were recently developed by Katzenellenbogen and colleagues to determine the roles of ERα and ERβ in mediating the effects of estrogens on food intake and body weight gain. 4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT) is an ERα receptor agonist that displays a 410-fold selectivity for ERα over ERβ [17]. 2,3-Bis(4-hydroxyphenyl)-propionitrile (DPN) is an ERβ receptor agonist that shows 70-fold selectivity for ERβ over ERα [18]. These agonists have recently been used to differentiate the roles of ERα and ERβ in mediating estrogen-induced uterine growth [17,19]. Both of these compounds most likely cross the blood–brain barrier as subcutaneous administration of PPT has been shown to increase progesterone receptor mRNA expression in the arcuate and ventromedial hypothalamic nuclei [17] and subcutaneous administration of DPN has been shown to produce anti-anxiety behaviors in OVX rats [20]. Therefore, systemic administration of these compounds allowed investigation of the predominant role of stimulating both systemic and central ERα and ERβ receptors in mediating food intake and body weight gain. Based on studies showing that ERα and ERβ actions frequently oppose one another [21], it was hypothesized that systemic administration of either ERα or ERβ selective agonists would have opposite effects on food intake and body weight gain.

Cyclic injection of estradiol (E_2) every fourth day in OVX rats produces a near physiological cyclic pattern of plasma estradiol concentration and spontaneous feeding behavior and normalizes body weight gain to rates observed in intact animals [22]. Continuous administration of peak physiological doses of estrogen in OVX rats decreases food intake and body weight gain to a greater extent than that observed in intact rats [8,23]. The most physiological HRT regimens used in postmenopausal women include daily administration of an estrogen with addition of a progestin for 12–14 days per month [24]. However, in order to avoid the withdrawal bleeding that is experienced when the progestin sequence is completed, many women prefer continuous unopposed estrogen administration or continuous combined estrogen and progestin replacement therapy [24]. Because of this preference, this paper investigated the effects of continuous administration of E_2 and ER subtype selective agonists on food intake and body weight gain using OVX rats as a model of menopause.

2. Methods

2.1. Subjects

Female Sprague–Dawley rats (Harlan, Indianapolis, IN) initially weighing 220–275 g were housed individually in $54 \times 30 \times 20$ cm plastic cages with Cell-Sorb Plus bedding (Fengman Specialties, Inc., Cincinnati, OH) in a temperature-controlled room with a 12 h light cycle. Rats were typically supplied standard laboratory chow (Purina lab diet 5001, PMI Nutrition International, Brentwood, MO) except during experiments when rats were supplied with liquid AIN-76 (Bio-Serv, Frenchtown, NJ) as indicated in each protocol. A separate group of rats was used for each protocol described below.

Rats were sham-operated or OVX under isoflurane anesthesia. E_2 (Sigma, Allentown, PA), the selective ERα agonist, PPT (Tocris, Ellisville, MO) or the selective ERβ agonist, DPN (Tocris, Ellisville, MO), were administered by daily injection in order to mimic widely used continuous HRT regimens. Hormone treatment was begun the same day as ovariectomy in order to avoid alterations in peripheral and/or central sensitivity to estrogen due to exposure to an estrogen-deficient state. It is unclear whether exposure to estrogen-deficient state would increase or decrease sensitivity to the agonists. For example, one study showed that the ability of estrogen to down-regulate adrenal and renal angiotensin type 1 receptor (AT_1R) density is attenuated by previous exposure to an estrogen-deficient state [25]. In contrast, another study showed that estrogen decreases ERα protein expression in vivo and in