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Estrogen status alters tissue distribution and metabolism of selenium in female rats☆,☆☆

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Abstract

A reported association between estrogen and selenium status may be important in the regulation of selenium metabolism. In this study, the effect of estrogen status on the metabolism of orally administered ^{75}Se-selenite and tissue selenium status was investigated. Female Sprague–Dawley rats were bilaterally ovariectomized at 7 weeks of age and implanted with either a placebo pellet (OVX) or pellet containing estradiol (OVX+E2), or were sham operated (Sham). At 12 weeks of age, 60 μCi of ^{75}Se as selenite was orally administered to OVX and OVX+E2 rats. Blood and organs were collected 1, 3, 6 and 24 h after dosing. Estrogen status was associated with time-dependent differences in distribution of ^{75}Se in plasma, red blood cell (RBC), liver, heart, kidney, spleen, brain and thymus and incorporation of ^{75}Se into plasma selenoprotein P (Sepp1) and glutathione peroxidase (GPx). Estrogen treatment also significantly increased selenium concentration and GPx activity in plasma, liver and brain, selenium concentration in RBC and hepatic Sepp1 and GPx1 messenger RNA. These results suggest that estrogen status affects tissue distribution of selenium by modulating Sepp1, as this protein plays a central role in selenium transport.

Keywords: Selenium; Estrogen; Selenoprotein P; Glutathione peroxidase; Ovariectomized rats

1. Introduction

A relationship between estrogen status and selenium metabolism has been suggested. Selenium concentrations in blood [1,2], liver [3,4] and brain [5] have been reported to differ in adult male and female mammals, and blood glutathione peroxidase (GPx) activity declines during pregnancy in rats [6] and humans [7,8], suggesting that estrogen modulates selenium metabolism. Blood selenium and GPx activity were positively correlated with estrogen concentrations during the rat estrous cycle [9] and the human menstrual cycle [10]. Also, estrogen administration significantly increased erythrocyte GPx activity in both premenopausal [11] and postmenopausal women [12,13]. 17β-Estradiol administration increased the amount of GPx4 messenger RNA (mRNA), but not GPx1 mRNA or GPx3 mRNA, in the bovine oviduct [14]. Glutathione peroxidase mRNA expression in hepatoma H4IIE cells exposed to the dietary phytoestrogen daidzein was also increased by 40% [15], but not by the addition of 17β-estradiol to cultures of human endothelial cells [16].

Twenty-five human selenoproteins have been identified including multiple isoforms of GPx [17]. Selenoprotein P (Sepp1) has attracted much attention in light of its role in the transport of selenium from liver to other tissues [18,19]. Selenoprotein P synthesis is regulated by dietary selenium intake, as inadequate selenium is associated with decreased plasma Sepp1 in rats [20] and humans [21] and significantly lower Sepp1 mRNA levels in rat liver and kidney [22]. The potential influence of estrogen status on Sepp1 expression and activity has not been previously examined.

Sex-based differences in the health effects of selenium have been reported in a number of epidemiologic studies [23]. Results from prospective human studies indicate that anticarcinogenic effects of selenium are generally greater in men than in women [24], suggesting endocrine influences on the health-promoting effects of selenium. Although previous research has focused on the effect of estrogen status or estrogen administration on selenium concentration and GPx activity in plasma and erythrocytes, more specific investigations of the impact of estrogen on distribution and metabolism of selenium in multiple tissues are limited. The purpose of this study was to investigate the effects of estrogen status on the apparent absorption, tissue distribution and incorporation of selenium into selenoproteins. The effect of estrogen status on

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selenium status also was determined by determining selenium concentrations and GPx activity in different tissues, hepatic levels of Sepp1 mRNA and GPx mRNA and plasma Sepp1.

2. Materials and methods

2.1. Animals and diets

Female Sprague–Dawley (SD) rats (Taconic, Germantown, NY, USA) weighing 45–55 g were fed the casein-based AIN-93G diet containing 150 μg total Se/kg diet as sodium selenate (Research Diets, New Brunswick, NJ, USA) beginning at weaning (3 weeks) and throughout the study. At 7 weeks of age, rats were randomly divided into three groups. The first two groups underwent ovariectomy with (OVX+E2, n=22) or without (OVX, n=22) estrogen replacement. The third group was sham operated (Sham, n=24) and designed for six rats to be killed on each day of the estrous cycle. Estrous cycles were determined by examining vaginal smears as described previously [9]. Rats in the OVX+E2 group were implanted with a subcutaneous pellet containing 1.5 mg 17β-estradiol for release over 60 days (Innovative Research of America, Sarasota, FL, USA). Rats in the OVX and Sham groups were subcutaneously implanted with a placebo pellet. The animals were housed in individual plastic cages in a room with controlled temperature (20°C–22°C) and a 12-h light–dark cycle with lights off from 18:00 to 06:00 h. Animals were fed between 08:00 and 09:00 h. Because OVX+E2 rats had lower food intakes than the other groups, the OVX+E2 group was given free access to diet, and the OVX and Sham groups were pair-fed the mean intake of the OVX +E2 group during the previous day in order to maintain similar caloric and selenium intake among animals. Food intakes were recorded daily, and body weights were recorded weekly. Animals were fasted overnight before sacrifice. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of The Ohio State University.

2.2. Experimental design

The effect of estrogen on the metabolism of ^{75}Se-selenite was studied in the OVX +E2 and OVX groups because they were expected to have the higher and lower plasma concentrations of estrogen, respectively, than those for the Sham group. At 12 weeks of age, the OVX and OVX+E2 groups were administered 60 μCi of ^{75}Se-sodium selenite with specific activity of 1400 Ci/g (University of Missouri Research Reactor Facility, Columbia, MO, USA) in 1.0 ml 150 mM NaCl by gavage. Four rats in each group were killed 1, 3, 6 and 24 h after ^{75}Se administration. Rats were anesthetized by brief exposure to carbon dioxide prior to terminal cardiac puncture for collection of blood into syringes containing 10 mg Na_2EDTA in 100 μl saline. Liver, kidney, heart, brain, lung, thymus, spleen and the gastrointestinal (GI) tract (including esophagus, stomach, duodenum, small intestine, large intestine, rectum and the anus), and its contents were collected. Another set of rats from the OVX (n=6), OVX+E2 (n=6) and Sham (n=24) groups that did not receive ^{75}Se was also killed at 12 weeks of age to collect blood, liver, kidney, heart and brain for determination of total selenium content, GPx activity, hepatic levels of GPx and Sepp1 mRNAs, and plasma Sepp1. All samples were stored at −80°C before analysis.

2.3. Plasma 17β-estradiol and ceruloplasmin activity

Concentration of plasma 17β-estradiol was determined using a double-antibody estradiol ^{125}I radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, USA). Because the copper containing protein ceruloplasmin has been shown to be up-regulated by estrogen [25], ceruloplasmin enzyme activity served as a positive control for estrogen-mediated alteration of trace element metabolism. Ceruloplasmin activity was measured according to Schosinsky et al. [26] with o-dianisidine dihydrochloride as the substrate.

2.4. ^{75}Se in tissues, cytosol and membrane

^{75}Se was determined in plasma, red blood cell (RBC), and organs with a Cobra II auto-gamma counter (Packard Instrument Company, Meriden, CT, USA). As organs were not perfused during collection, contamination of erythrocytes and plasma was estimated via hemoglobin concentrations and subtracted from the totals in organs. For organs in which the ^{75}Se activity differed between the OVX and OVX+E2 groups, the distribution of ^{75}Se in total membrane (organelle) and cytoplasmic compartments was determined. Organs were homogenized in 50 mM phosphate buffer (25% homogenate) containing 10% sucrose, pH 6.3, using a Brinkman Polytron homogenizer (Brinkman Instruments Co., Westbury, NY, USA). An aliquot of the homogenate was centrifuged at 4°C for 90 min at 110,000g (Ti 50 rotor, Beckman Model L7-65, Palo Alto, CA, USA), and supernatant (cytosol) was removed to measure ^{75}Se activity. Activity was normalized by protein concentration determined by the bicinchoninic acid method (BCA protein assay kit, Pierce, Rockford, IL, USA). ^{75}Se activity in the membrane fraction was estimated by subtracting activity in cytosol from the total activity in homogenate.

2.5. Determination of ^{75}Se in selenoproteins

The incorporation of ^{75}Se into selenoproteins was determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Proteins in plasma and cytosol were separated with 5% stacking and 12% resolving gels. Ten-microliter samples containing 50–100 μg protein were loaded to each well. Gels were sliced according to the known molecular weights of well-characterized selenoproteins, and the ^{75}Se activity in gel slices was measured by gamma ray spectrometry (Packard Cobra II Auto Gamma; Packard Instrument Company, Meriden, CT). This procedure provides a convenient method to examine the ^{75}Se distribution among selenoproteins without the use of antibodies and has been used to identify selenoproteins with molecular weights from 12 to 75 kDa [27]. Total protein ^{75}Se was defined as the sum of ^{75}Se activity of every band with molecular weight between 12 and 75 kDa. Here we investigated the distribution of ^{75}Se into Sepp1 and GPx by using this method.

2.6. Selenium status

Selenium concentrations in diet, plasma, RBC, liver, kidney, heart and brain were measured by the gas chromatography technique of McCarthy et al. [28] using an Agilent 6890 Series gas chromatograph with a 225 Durabond Megabor column and an electron capture detector maintained at the following temperatures: oven (column) 190°C, front inlet (injector) 220°C and front detector 300°C. Nitrogen (~60 psi) and helium (~80 psi) were used as carrier gases. Data were integrated using an Agilent 6890 Series Integrator. Glutathione peroxidase activity of plasma, RBC, liver, kidney, brain and heart was determined by the coupled assay of Paglia and Valenine [29] using a Shimadzu UV-visible recording spectrophotometer (model UV160U). The activity was expressed as units per gram of protein (hemoglobin for RBC). One unit of activity is equivalent to 1 μmol NADPH oxidized per minute at 37°C. The protein concentration in plasma and cytosol was measured by the bicinchoninic acid method (Pierce, Rockford, IL, USA) with the Shimadzu UV-visible recording spectrophotometer (model UV160U). The hemoglobin concentration of RBC was determined with the Shimadzu UV-visible recording spectrophotometer (model UV160U) using Drabkin's reagent. Plasma Sepp1 concentration was determined by a competitive radioimmunoassay using a modified method of Hill et al. [30].

2.7. Hepatic levels of Sepp1 mRNA and GPx1 mRNA

Total RNA was extracted from the liver of rats in the OVX (n=6) and OVX+E2 (n=6) groups using a RNAqueous-4PCR kit (Ambion, Austin, TX, USA). First-stand complementary DNA (cDNA) was synthesized from 1 μg RNA samples using the SuperScript First-Strand Synthesis System for reverse transcriptase polymerase chain reaction (RT-PCR) (Invitrogen, Carlsbad, CA, USA). To quantify the cDNA of Sepp1 and GPx1 mRNA, quantitative real-time PCR analysis was performed in an Mx3000P QPCR system (Stratagene, La Jolla, CA, USA) using the published primers for Sepp1 [31], GPx1[32] and GAPDH [33]. Temperature cycling conditions were 95°C for 2 min, 45 cycles of 95°C for 30 s and 60°C for 1 min. The amounts of GAPDH mRNA were used to normalize expression.

2.8. Statistical methods

Data were reported as means±S.E. The Student's t test was used to detect the differences between OVX+E2 and OVX groups. Analysis of variance was utilized to determine the differences of the indices at different times after administering ^{75}Se and to determine the differences of the indices among treatment groups in the nonradioactive study. Differences were considered significant at P<.05. When significant difference among the means was indicated, Tukey's post hoc probability test was performed to determine which means differed significantly. All statistical analyses were performed using SPSS V18 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Body weights

Body weights for the three experimental groups prior to the ovariectomy and sham operations at 7 weeks of age were not significantly different (P>.05, Fig. 1). Chronic administration of estrogen to ovariectomized rats (OVX+E2) decreased body weight gain during the first postoperative week. Rats in the OVX group had the most rapid increase in body weight, followed by the Sham group and then the OVX+E2 group despite using a pair-feeding paradigm. At weeks 8–10, body weights of the OVX and Sham groups were not significantly different, but significantly greater than that of the OVX+E2 group (P<.05). At weeks 11 and 12, body weight differed significantly among the three groups with OVX>-Sham>OVX+E2 (P<.05).