All Regions of Mouse Brain Are Dependent on Selenoprotein P for Maintenance of Selenium

Akihiro Nakayama,1 Kristina E. Hill, Lori M. Austin, Amy K. Motley, and Raymond F. Burk*

Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232-0232

Abstract

The brain and testis retain selenium better than other tissues during selenium deficiency. Studies of mice with selenoprotein P (Sepp1) deleted (Sepp1−/− mice) showed that brain and testis selenium levels are largely dependent on Sepp1. Therefore, we examined tissue selenium in mice fed varying amounts of selenium and in Sepp1+/− mice to characterize better the role(s) of Sepp1. Mice were fed a selenium-deficient diet for 8 wk supplemented with selenium as selenite from none to 0.25 mg/kg diet and tissue selenium was measured. Brain and testis maintained their selenium better than did liver, kidney, and muscle when dietary selenium was limiting but testis selenium fell sharply in the group fed the deficient diet. Brain retained its selenium well, even in the group fed the deficient diet. After intravenous injection of 75Se-Sepp1 into Sepp1+/− and Sepp1−/− mice, qualitative differences between brain and testis 75Se uptake were noted, further suggesting differences in their uptake of selenium from Sepp1. Finally, selenium was measured in brain regions of Sepp1−/− and Sepp1+/− mice fed the diet supplemented with 1 mg selenium/kg and Sepp1−/− mice fed the deficient diet. Deletion of Sepp1 and selenium deficiency each lowered selenium a similar amount in cortex, midbrain, brainstem, and cerebellum. Selenium in the hippocampus was lowered by deletion of Sepp1 but not by selenium deficiency. These results suggest that Sepp1 is more important for maintaining selenium in the hippocampus than in other brain regions. They also confirm the position of the brain at the apex of the organ selenium hierarchy. J. Nutr. 137: 690–693, 2007.

Introduction

Selenium is a micronutrient that derives its essentiality from selenoproteins that have redox functions. The glutathione peroxidases and thioredoxin reductases regulate thiol redox status in cells and are responsible for much of the antioxidant effect of the element. Another selenoprotein family, the iodothyronine deiodinases, regulates thyroid hormone metabolism. Functions of a number of selenoproteins remain to be determined.

For many years, the only human disease proven linked to selenium deficiency has been Keshan disease, a childhood cardiomyopathy reported from selenium-deficient regions of China (1). Now, with the identification of proteins that are responsible for the disposition of selenium, including synthesis of selenoproteins, it is becoming possible to identify diseases caused by protein abnormalities that impair selenoprotein synthesis (2).

Homeostasis of micronutrient minerals that have redox properties ensures supply of those elements at sites of function and avoids accumulation of them, both conditions that lead to metabolic dysfunction and tissue damage. Abnormalities of the proteins that effect homeostasis of these minerals cause a variety of pathological conditions ranging from Menke’s disease to hemochromatosis. Identification and characterization of the proteins essential for selenium homeostasis are needed so that abnormalities in them can be sought as disease causes.

Selenoprotein P (Sepp1) is involved in maintaining selenium homeostasis. It is an extracellular protein that contains most of the selenium in plasma (3). All tissues appear to express Sepp1, but the liver is the principal source of it in plasma (4).

Sepp1 is involved in the distribution of selenium to tissues (5) and in the maintenance of whole-body selenium homeostasis (6). Mice with deletion of the Sepp1 gene (Sepp1−/− mice) have sharply depressed brain and testis selenium concentrations (5) and feeding them a low-selenium diet causes central nervous system dysfunction and death (7).

The major objective of the studies reported here was to determine whether Sepp1 affects selenium differently among brain regions. Experiments were carried out to examine the effect of dietary selenium supply and of deletion of Sepp1 on selenium concentrations in several tissues and in regions of the brain.

Materials and Methods

Animals. Congenic (C57BL/6) Sepp1−/− breeding pairs were mated to produce Sepp1−/− and Sepp1+/− male mice (7). Weanling Sepp1+/− and Sepp1−/− mice were fed a Torula yeast-based diet supplemented with selenium as sodium selenite (7). The selenium-deficient diet was the same diet without selenium supplementation. Additional C57BL/6 male weanling mice, purchased from the Jackson Laboratory, were fed the...
experimental diets. The Torula yeast diets were formulated and pelleted to our specifications by Harlan-Teklad. The mice had free access to food and tap water. The light cycle in the animal room was 10 h light and 14 h dark. The Vanderbilt University Institutional Animal Care and Use Committee approved the studies described herein.

**Selenium-repletion study.** Weanling C57BL/6 male mice were fed the selenium-deficient diet for 18 wk, after which groups of mice were formed and fed selenium-supplemented diets. The selenium supplements in the diets were 0, 0.025, 0.05, 0.075, 0.10, and 0.25 mg selenium/kg diet. Under the dietary conditions used in this experiment, virtually all the selenium in the mice was expected to be present in the form of selenoproteins. After 8 wk, 3 mice from each group were studied. Each mouse was anesthetized with isoflurane prior to exsanguination by removal of blood from the inferior vena cava. The blood was treated with EDTA (1 g/L) to prevent clotting. Liver, kidney, muscle, brain, and testis were harvested and immediately frozen in liquid N2. Plasma was obtained by centrifugation of the blood at 16,000 × g for 2 min. Plasma and tissues were stored at −80°C until assayed for glutathione peroxidase activity, Sepp1, and selenium.

**75Se-Sepp1 injection study.** 75Se-Sepp1 was prepared for administration by injecting a tracer dose (10 μCi) of 75Se-selenite into a mouse with deletion of glutathione peroxidase-3. The 75Se-selenite (900 Ci/g selenium) had been purchased from the University of Missouri Research Reactor Facility. After 3 h, the mouse was exsanguinated from the vena cava under anesthesia and serum was obtained. The serum was dialyzed against PBS. The dialyzed serum was sterilized with a 0.2-μm filter before injection of 200 μL into the tail vein of another mouse. At 5, 10, 30, 60, 120, and 240 min after injection, mice were anesthetized and exsanguinated from the vena cava. The blood was treated with EDTA (1 g/L) to prevent clotting. Liver, kidney, muscle, brain, and testis were harvested and immediately frozen in liquid N2. Plasma was obtained by centrifugation of the blood at 16,000 × g for 2 min. Plasma and tissues were stored at −80°C until assayed for glutathione peroxidase activity, Sepp1, and selenium.

**Distribution of selenium in brain regions.** Sepp1−/− and Sepp1+/+ mice were fed the experimental diets from weaning. Sepp1+/+ mice were fed the selenium-deficient diet or the same diet containing 1 mg selenium as selenite/kg (high-selenium diet). Sepp1−/− mice were fed the high-selenium diet. Mice were fed the diets for 8 to 12 wk except for 1 group of 4 Sepp1−/− mice fed the selenium-deficient diet that constituted a single sample. Mice in this group were fed the diet for 30 wk.

After exsanguination under anesthesia, the brain was removed, weighed, and dissected into cerebral cortex, brainstem, midbrain, cerebellum, and hippocampus (8). Brain regions from 4 mice were pooled for each sample. Three samples of each brain region were assayed for selenium. Thus, 12 mice were studied in each of the 3 groups.

**Assays.** Plasma glutathione peroxidase activity and plasma Sepp1 concentration were determined, as previously described (5). Selenium was measured using the method of Koh and Benson (9), as modified by Sheehan and Gao (10).

**Statistics.** Data were reported as mean ± SD. Statistical analyses were performed using Newman-Keuls Multiple Comparison test following 1-way ANOVA analysis. P < 0.05 was considered significant. All calculations were performed using GraphPad Prism version 4.0b on an Apple Macintosh G5.

**Results**

When selenium was fed in an amount adequate for full expression of all selenoproteins (0.25 mg/kg diet), its concentration varied among tissues (Fig. 1). Kidney, liver, and testis had higher selenium concentrations than did brain and muscle. The results in Figure 1 provide the reference points for selenium concentrations in tissues of mice fed suboptimal amounts of selenium.

Feeding amounts of selenium that did not allow full expression of selenoproteins revealed different patterns of selenium concentration from tissue to tissue (Fig. 2A). Liver, kidney, and muscle had similar patterns, with the decrease in their selenium concentrations roughly proportional to the decrease in selenium fed to the mice. The plasma selenium biomarkers, Sepp1 and glutathione peroxidase (Fig. 2B), mirrored liver, kidney, and muscle selenium concentrations.

Brain and testis maintained their selenium better than did liver, kidney, and muscle when dietary selenium became limiting (Fig. 2A). Testis selenium was maintained except in mice fed the...
basal diet with no supplemental selenium. In that extreme selenium-deficiency state, testis selenium fell to 9% of the value determined in the adequately supplemented mice.

Brain selenium decreased the least of any tissue, to 74%, in mice fed the diet with no selenium supplementation. Thus, brain is able to maintain its selenium at the expense of other mouse tissues, including testis. These results confirm and extend previous reports (5,11).

Sepp1−/− mice have sharply decreased brain and testis selenium concentrations, so the fate of selenium in Sepp1 was examined in them and in Sepp1+/+ mice. Sepp1−/− mice were fed a high-selenium diet to avoid central nervous system injury (7); groups of Sepp1+/+ mice were fed the same high-selenium diet or the selenium-deficient diet. After injection of 75Se-labeled Sepp1 by tail vein, plasma 75Se decreased over 4 h in a pattern that was similar in all 3 groups (Fig. 3). The half-life of 75Se in plasma, based on the 1-h and 4-h values of all mice studied, was ~8 h. The groups did not differ in 75Se in liver.

Brain 75Se rose steadily over the 4-h observation period in Sepp1−/− mice fed a high-selenium diet and in Sepp1+/+ mice fed a selenium-deficient diet (Fig. 3). Sepp1+/+ mice fed a high-selenium diet did not accumulate brain 75Se during the 4-h period. Thus, the 2 groups with low Sepp1 levels (albeit for different reasons) had similar brain 75Se uptakes that were greater than the uptake in the group with higher Sepp1 levels. This result is compatible with regulated uptake of Sepp1-selenium by the brain.

Testis 75Se was similar in the 3 groups for the first 2 h, but at the 4-h time point, the 2 groups with low Sepp1 had higher values than the Sepp1+/+ mice fed a high-selenium diet. Thus, the pattern of uptake by the testis was qualitatively different from that of the brain (see Fig. 3).

Because brain selenium is so highly protected by the organism, selenium was measured in major brain regions. Selenium concentration was decreased to a similar extent in midbrain, cortex, and cerebellum by deletion of Sepp1 and by feeding a selenium-deficient diet (Fig. 4).

Figure 3 Changes in 75Se in plasma and tissues after intravenous administration of 75Se-Sepp1 to male Sepp1−/− and Sepp1+/+ mice. Zero Se represents Sepp1−/− mice fed selenium-deficient diet. The other mice were fed the high-selenium diet. Two mice were studied at each time point and the lines were drawn through the means. Both values are shown at the 240-min time point to indicate variation.

Selenium concentrations in brain regions of male Sepp1−/− and Sepp1+/+ mice. Zero Se represents Sepp1−/− mice fed selenium-deficient diet. The other mice were fed the high-selenium diet. Bars are means ± SD, n = 3. * Different from other means in that brain region, P < 0.05. Whole-brain concentrations, calculated by totaling selenium contents of all regions and dividing by brain weight, were: Sepp1+/+ fed selenium-deficient diet, 86 ± 12 ng/g; Sepp1−/− mice fed high-selenium diet (1 mg selenium/kg), 91 ± 8 ng/g; and Sepp1+/+ mice fed high-selenium diet, 136 ± 9 ng/g. One mol Se = 79 g.

Figure 4 Selenium concentrations in brain regions of male Sepp1−/− and Sepp1+/+ mice. Zero Se represents Sepp1−/− mice fed selenium-deficient diet. The other mice were fed the high-selenium diet. Bars are means ± SD, n = 3. * Different from other means in that brain region, P < 0.05. Whole-brain concentrations, calculated by totaling selenium contents of all regions and dividing by brain weight, were: Sepp1+/+ fed selenium-deficient diet, 86 ± 12 ng/g; Sepp1−/− mice fed high-selenium diet (1 mg selenium/kg), 91 ± 8 ng/g; and Sepp1+/+ mice fed high-selenium diet, 136 ± 9 ng/g. One mol Se = 79 g.

**Discussion**

Studies of mice with deletion of Sepp1 indicate that brain and testis derive much of their selenium from this selenoprotein (5,12). We have speculated that selenium uptake by these tissues might be via receptors for Sepp1 (3) and we have obtained results to support such a mechanism (G. E. Olson, V. P. Winfrey, S. K. NagDas, K. E. Hill, and R. F. Burk, unpublished data).

Brain and testis have extraordinary abilities to maintain selenium concentrations under selenium-deficient conditions. However, Figure 2A shows that brain maintains its selenium in the most severe selenium deficiency even better than does testis. Moreover, the pattern of 75Se uptake from 75Se-labeled Sepp1 by brain appears to be qualitatively different from that of testis (Fig. 3). These results are compatible with receptor mechanisms of selenium uptake by brain and testis, but they also demonstrate that there are differences between these 2 tissues in selenium uptake characteristics. Sepp1 is expressed throughout the brain (13,14) and its role there has been postulated to be as a local storage and transport form of selenium (15). Comparable expression is not observed in the testis (16). Such storage and transport roles might account for the superior ability of the brain to retain selenium. However, identifying the receptors involved in Sepp1 uptake from the blood will allow a better understanding of selenium metabolism in the 2 tissues.

It seemed possible that particular regions of the brain might metabolize selenium differently from other regions. The results in Figure 4 show that the hippocampus maintained its selenium under deficient conditions better than did other major brain regions. However, hippocampal selenium concentration was dependent on Sepp1, as was selenium in other brain regions. These 2 findings suggest that selenium and Sepp1 are especially
important to the hippocampus, which facilitates memory and learning. A recent study (17) has shown impaired spatial learning in Sepp1−/− mice, even though they were fed a high-selenium diet. Brain slices from the Sepp1−/− mice demonstrated severely altered hippocampal synaptic transmission, short-term plasticity, and long-term potentiation. These results suggest that synaptic transmission is impaired in the hippocampus when Sepp1 is not present and that this leads to impairment of at least one type of learning.

Sepp1 metabolism differs between species. After injection of 75Se-Sepp1 into selenium-replete animals, plasma 75Se disappeared more slowly in mice (Fig. 3) than in rats (18). There was a distribution phase during the first hour, indicating that 75Se (presumably in the form of 75Se-Sepp1) was distributed to the tissues from the blood. The half-life, based on the 75Se values from 1 to 4 h, was ~8 h in mice, whereas it has been determined to be 3–4 h in rats (18). Thus, Sepp1 turns over rapidly in both species but slightly more rapidly in rats than in mice.

The rapid turnover in plasma is compatible with rapid consumption of Sepp1 in the periphery. Although uptake by testis and brain seems certain, that alone would not account for such rapid disappearance of Sepp1. Thus, the rapid consumption is likely to reflect breakdown in other tissues for delivery of selenium and/or for some other, as yet undiscovered, function.

In conclusion, results of this study confirm that brain is at the apex of the organ hierarchy of selenium retention and that testis is just beneath brain (Fig. 2A). Both these organs had strikingly different responses to selenium deficiency than did liver, kidney, and muscle, compatible with their uptake of selenium from Sepp1 being mediated by receptors. Within the brain, the hippocampus resisted selenium depletion except when Sepp1 was absent, indicating a special function of Sepp1 in this brain region.

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Literature Cited