Neurological Dysfunction Occurs in Mice with Targeted Deletion of the Selenoprotein P Gene1,2

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ABSTRACT Brain function and selenium concentration are well maintained in rodents under conditions of selenium deficiency. Recently, however, targeted deletion of the selenoprotein P gene (Sepp) has been associated with a decrease in brain selenium concentration and with neurological dysfunction. Studies were conducted with Sepp−/− and Sepp+/+ mice to characterize the neurological dysfunction and to correlate it with dietary selenium level. When weanling Sepp−/− mice were fed the basal diet (<0.01 mg/kg selenium) supplemented with 0, 0.05 or 0.10 mg selenium/kg, they developed spasticity that progressed and required euthanasia. Supplementing the diet with ≥0.25 mg selenium/kg prevented the neurological dysfunction. To determine whether neurological dysfunction would occur in more mature Sepp−/− mice deprived of selenium, Sepp−/− mice that had been fed the basal diet supplemented with 1.0 mg selenium/kg for 4 wk were switched to a selenium-deficient diet. Within 3 wk they had developed neurological dysfunction and weight loss. At 3 wk, the 1.0 mg selenium/kg diet was reinstituted. Neurological function stabilized but did not return to normal. Brain selenium concentration did not increase. Weight gain resumed. This study shows that neurological dysfunction occurs when selenium supply to the brain is curtailed and that the dysfunction is not readily reversible. Both the absence of selenoprotein P and a low dietary selenium supply are necessary for the dysfunction to occur, indicating that selenoprotein P and at least one other form of selenium supply the element to the brain. J. Nutr. 134: 157–161, 2004.

KEY WORDS: • deletion of selenoprotein P • mouse selenoprotein P • neurological dysfunction • selenium transport to brain

Neurological function is well maintained in selenium-deficient rats and mice. The likely reason for this is that the concentration of selenium in the brain remains close to normal when the element is in short supply even while the selenium concentrations of other tissues decline drastically (1). Just how the brain is able to concentrate selenium in an animal with an insufficiency of the element has not been elucidated.

Over a decade ago, our group showed that selenoprotein P, a selenium-rich plasma protein derived largely from the liver, delivered selenium to the brain (2). In addition, we observed that brain uptake of selenium from this protein was upregulated in selenium-deficient rats, whereas uptake by other tissues was not. These findings led to the hypothesis that brain selenium is maintained under conditions of varying selenium supply by regulated acquisition from selenoprotein P.

Recently, a German group and our group independently produced mice with deletion of the selenoprotein P gene (Sepp) (3,4). Both groups observed that the Sepp−/− mice had lower brain selenium concentrations than Sepp+/+ and Sepp+/− mice and that they developed neurological dysfunction. Our group demonstrated that feeding selenium at dietary levels above the usual mouse requirement of 0.10 mg/kg prevented development of the dysfunction (3).

This report describes and characterizes the neurological dysfunction that occurs in the Sepp−/− mice. It also correlates the neurological dysfunction with dietary selenium supply.

MATERIALS AND METHODS

Animals. Because Sepp−/− male mice have low fertility, Sepp−/− male and female mice were mated and their offspring were genotyped (3). Male Sepp+/− and Sepp−/− offspring were selected at weaning for study. They were fed a Torula yeast-based diet that contained <0.01 mg selenium/kg (Table 1) or the same diet with selenium added. The mouse selenium requirement is 0.10 mg/kg (5,6). Selenium was added to the diet as sodium selenite or as L-selenomethionine (a gift from Dr. V. Badmaev, Sabinsa, Piscataway, NJ). As this study was progressing, the original Sepp+/− female breeders were being mated with C57Bl/6 males (Jackson Laboratories, Bar Harbor, ME) to obtain a congenic strain. The original Sepp+/− breeding colony was on an SV129/C57Bl/6 genetic background. The offspring of the original breeders were designated BCl (backcross1); subsequent generations from matings of Sepp+/− females with C57Bl/6 males were designated...
analyses were performed by Student’s t test or by using Fisher’s Protected Least Significant Difference and Scheffe’s post-hoc test after analysis with repeated-measures ANOVA. Probability values < 0.05 were considered to indicate significant differences. All calculations were performed using Statview 5.0.1 (SAS Institute, Cary, NC) on an Apple Macintosh G4.

### TABLE 1

<table>
<thead>
<tr>
<th>Component</th>
<th>g/kg diet</th>
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<tbody>
<tr>
<td>Torula yeast1</td>
<td>300</td>
</tr>
<tr>
<td>Sucrose</td>
<td>568.3</td>
</tr>
<tr>
<td>Corn oil</td>
<td>66.7</td>
</tr>
<tr>
<td>Mineral mix2</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mix3</td>
<td>10</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
</tr>
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</table>

1 Lakes States Torula Dried Yeast, U.S.P. XIX, Type B, supplied by Lake States Division, Rhinelander Paper, Rhinelander, WI.
2 Contains (g/kg) CaCO, 543; KH2PO4, 225.2; KCl, 104.8; NaCl, 59.68; MgCO3, 25; MgSO4, 16; ferric ammonium citrate, 20.5; MnSO4·H2O, 3.44; NaF, 1; CuSO4·6H2O, 0.1; KI, 0.08.
3 ALN-76A vitamin mix (15) supplied by ICN, Aurora, OH.

RESULTS

**Neurological dysfunction in young Sepp<sup>−/−</sup> mice.** Weaning Sepp<sup>−/−</sup> mice fed a selenium-deficient diet or the same diet supplemented with 0.05 or 0.10 mg selenium/kg developed signs of neurological dysfunction (Table 2). No Sepp<sup>+/−</sup> or Sepp<sup>+/+</sup> mice exhibited any of these signs, even when fed a selenium-deficient diet for a prolonged period (3).

Diverse abnormalities were observed. A wide stance was present in most cases. Some mice appeared to slither along on their undersides with the appearance of swimming. Others maintained their bodies above the surface on extended legs. Some only moved backward. As the impairment progressed, episodic poorly coordinated hyperactivity was observed in which running mice struck the side of the cage. When placed on a table, such mice would bolt over the side. Mice became unable to right themselves from a lying position on a smooth surface. Finally, their spasticity became extreme with extended limbs and they became unable to walk.

**Assessment of skeletal muscle injury of affected mice.** Skeletal muscle from Sepp<sup>−/−</sup> mice that had been fed a selenium-deficient diet until they had developed spasticity and from matched Sepp<sup>+/−</sup> mice was examined by light microscopy and plasma creatine phosphokinase activity was measured. No evidence of muscle injury was found by either method (data not shown).

**Quantitation of neurological impairment in mice fed different amounts of selenium from weaning.** Weaning Sepp<sup>−/−</sup> mice and Sepp<sup>+/−</sup> mice were fed the experimental diet supplemented with 0, 0.10, 0.25, 0.50 and 1.0 mg selenium/kg in the form of selenite or of selenomethionine. Selenite is an inorganic form of selenium frequently used in supplements and selenomethionine is the major organic form present in natural foodstuffs.

Table 3 presents the 4-week survival and weight gain of the mice. None of the Sepp<sup>−/−</sup> mice fed the selenium-deficient diet survived for 4 wk. All of them developed severe neurological impairment and lost weight, requiring that they be euthanized. When Sepp<sup>−/−</sup> mice were fed the 0.10 mg selenium/kg diet, almost half the group fed selenomethionine developed neurological dysfunction and weight loss requiring euthanasia, whereas none fed selenite developed either of

### TABLE 2

<table>
<thead>
<tr>
<th>Chronology</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Wide stance, waddling gait, walking backward</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Stiff gait, hopping gait, tense rear legs, uncoordinated running episodes, unable to right on smooth surface</td>
</tr>
<tr>
<td>Terminal</td>
<td>Stiff rear legs that are crossed, episodic hyperactivity, unable to walk</td>
</tr>
</tbody>
</table>

1 Exhibited by all mice fed diet not supplemented with selenium (n = 8) by 7 d and by all mice supplemented with 0.05 mg/kg selenium (n = 14) by 37 d. Nine of 12 fed 0.10 mg/kg selenium exhibited abnormalities by the end of the observation period of 56 d. The form of selenium added to the diet was selenite. See the supplemental movie online for demonstration of their movements.
mice fed 0.1 mg selenium/kg as selenomethionine developed overt neurological dysfunction before 4 wk (Table 3). No impairment of neurological function was detected in Sepp<sup>−/−</sup> mice, even when they were fed the selenium-deficient diet. These results indicate that deletion of selenoprotein P raises the dietary selenium requirement to maintain normal neurological function.

We observed Sepp<sup>−/−</sup> (n = 11) and Sepp<sup>+/+</sup> (n = 8) mice fed from weaning a diet supplemented with 1.0 mg selenium/kg as selenite for 15 mo. None developed signs of neurological dysfunction and stride lengths of the two groups were similar (data not shown). Thus, the effect of feeding high selenium is durable with respect to neurological function.

**Neurological function in adult Sepp<sup>−/−</sup> mice fed a selenium-deficient diet.** Because weanling Sepp<sup>−/−</sup> mice developed severe neurological impairment when fed a low selenium diet, we performed an experiment to test whether mice would be similarly affected when fed the selenium-deficient diet after the early period of rapid brain development that occurs around the time of weaning.

**Figure 3A** shows the design of the experiment. Both groups gained a similar amount of weight when fed the high selenium diet for the 4 wk following weaning. However, when the mice were then switched to the selenium-deficient diet for 3 wk, the Sepp<sup>−/−</sup> mice lost weight compared with the Sepp<sup>+/+</sup> mice, which continued to gain. Reinstitution of the high selenium diet led to similar weight gains of the two groups over the next 6 wk. The amount of selenium in the brains of the two groups diverged when the selenium-deficient diet was fed and did not reconverge when the high selenium diet was reinstated (Fig. 3B). Glutathione peroxidase activity was lower in brains of Sepp<sup>−/−</sup> mice under all conditions (Fig. 3C).

### TABLE 3

<table>
<thead>
<tr>
<th>Selenium added to diet</th>
<th>Genotype</th>
<th>Weight gain&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Survival&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Form</td>
<td>Weight gain&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Survival&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>g</td>
<td>Sepp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Sepp&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>Sepp&lt;sup&gt;−/−&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>Selenite</td>
<td>13 ± 2.0</td>
<td>7/7</td>
</tr>
<tr>
<td>0.10 mg/kg</td>
<td>Selenomethionine</td>
<td>6.2 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/7&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Selenite</td>
<td>11 ± 1.9</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Selenomethionine</td>
<td>12 ± 1.9</td>
<td>8/8</td>
</tr>
</tbody>
</table>

1 Mice were fed diets supplemented with 0, 0.10, and 0.25 mg selenium/kg. Other groups were administered 0.50 and 1.0 mg/kg selenium and they all survived with comparable weight gains (data not shown).

2 Values are means ± sd and n is shown in the next column as survivors. Values with the superscript “a” differ from one another by Student’s t test, P < 0.05.

3 The number of survivors is shown on the left and total mice in the group is shown on the right. The 3 animals that were euthanized were developed neurological impairment and weight loss.

4 All 10 mice gained weight for 14 d (5.4 ± 1.8 g) but developed severe neurological impairment and lost weight between 14 and 28 d, so that none survived to 28 d.

5 Three of the 7 mice developed severe neurological impairment and lost weight, requiring that they be euthanized before 28 d. The 4 remaining mice exhibited no obvious neurological impairment.

Neurological function was assessed in Sepp<sup>−/−</sup> and Sepp<sup>+/+</sup> mice by 3 standardized tests (stride length, pole climb, and rotarod). The stride pattern of a Sepp<sup>+/+</sup> mouse and that of a Sepp<sup>−/−</sup> mouse fed selenium-deficient diets differed (Fig. 1) in that the stride of the Sepp<sup>−/−</sup> mouse was shorter than that of the Sepp<sup>+/+</sup> mouse. The neurological function of these mice was tested weekly. Sepp<sup>−/−</sup> mice had impaired performance on all three tests when fed a diet supplemented with the mouse selenium requirement of 0.10 mg/kg for 4 wk beginning at weaning (Fig. 2). Raising the dietary selenium supplement to 0.25 mg/kg prevented development of the impairment. Sepp<sup>−/−</sup> and Sepp<sup>+/+</sup> mice fed 0.1 mg/kg differed significantly but those fed ≥ 0.25 mg/kg did not. Similar results were obtained whether sodium selenite or selenomethionine was the form of selenium added to the diet, although 3 of the 7 mice fed sodium selenite and 1 of the 7 fed selenomethionine exhibited no obvious neurological impairment.
Neurological tests were begun just before the feeding of the selenium-deficient diet was started (Fig. 4). The performance of the Sepp<sup>+/+</sup> mice deteriorated when they were fed the selenium-deficient diet, and refeeding the high selenium diet for 6 wk did not reverse this dysfunction.

**DISCUSSION**

This study documents the neurological dysfunction that occurs in weanling mice when they lack selenoprotein P and are fed a diet containing selenium at or below the recognized dietary requirement of 0.10 mg/kg. Either the presence of selenoprotein P or the feeding of a diet containing selenium in a concentration ≥0.25 mg/kg prevented the dysfunction (Fig. 2). These findings suggest that selenoprotein P supplies selenium to the brain (to prevent dysfunction) and that an increased dietary selenium supply can substitute for selenoprotein P in this respect.

Earlier, we reported the effect of dietary selenium on brain selenium concentration in Sepp<sup>−/−</sup> mice (3). At a dietary selenium concentration of 0.10 mg/kg, the brain selenium concentration in Sepp<sup>−/−</sup> mice was 43% of that in Sepp<sup>+/+</sup> mice. That is lower than can be produced by selenium deficiency alone (1) and is consistent with the hypothesis that selenoprotein P supplies selenium to the brain. However, raising the dietary selenium to 0.25 mg/kg did not increase whole-brain selenium concentration [see Fig. 5 in (3)], even though it did prevent neurological dysfunction. Thus, the prevention of neurological dysfunction by dietary selenium did not correlate with an observable increase in whole-brain selenium concentration. Although other explanations for these findings are possible, it seems most likely that raising dietary selenium increased selenium in a small, but critical, brain compartment and that this increase was not reflected in the total brain selenium concentration.

Selenium was fed as selenomethionine, the major form of selenium present in normal human food, and as selenite, a form of selenium often used in supplements, to assess the relative bioavailability of these forms. Selenium prevented weight loss better than did selenomethionine when each was fed at 0.10 mg selenium/kg (Table 3). Because both forms are efficiently absorbed, it seems likely that their difference in bioavailability is related to sequestration of some of the selenomethionine in the methionine pool, rendering its selenium unavailable for incorporation into selenoproteins (10). Thus, once expansion of the methionine pool, which is caused by growth of the mouse, has ceased, release of selenium from the selenomethionine in the methionine pool should equal its intake and selenomethionine bioavailability would be predicted to become equivalent to that of selenite.

The neurological dysfunction that occurs in the Sepp<sup>−/−</sup> mice is a progressive deterioration of motor function that is manifested as spasticity [Table 2 and online video (available as supplemental material in the online posting of this paper at www.nutrition.org)]. If selenium is not supplied, affected mice become unable to walk and then die. Neurological problems of this type are often traced to lesions in motor neuron tracts. Established neurological dysfunction is not readily reversible by resupply of dietary selenium (Fig. 4), suggesting that the injury to the nervous system in these mice results in loss of neurons.

After mice had been fed 1.0 mg selenium/kg for 4 wk, brain selenium concentrations were not different between Sepp<sup>−/−</sup> and Sepp<sup>+/+</sup> mice (Fig. 3B). However, brain glutathione peroxidase activities (Fig. 3C) were different between them. This signals that brain selenium does not correlate with brain glutathione peroxidase (and perhaps with other selenoproteins) in Sepp<sup>−/−</sup> mice. Such a discordance indicates that selenoprotein P and other transport forms of selenium are not equivalent with respect to the brain.

**FIGURE 2** Neurotesting of Sepp<sup>+/+</sup> mice (open bars) and Sepp<sup>−/−</sup> mice (filled bars) presented in Table 3. These mice had been fed diets containing different amounts of selenium as selenite for 4 wk from weaning. Values are means ± SD, n = 7–8. Means in a panel without a common letter differ (P < 0.05) by Fisher’s Protected Least Significant Difference post hoc test after repeated-measures ANOVA.

**FIGURE 3** Effect of feeding a selenium-deficient diet on body weight (A), brain selenium (B) and brain glutathione peroxidase activity (C) of Sepp<sup>−/−</sup> and Sepp<sup>+/+</sup> mice that had been fed a high selenium diet from weaning (0 wk). Values are means ± SD, n = 3–4 at 4 and 7 wk and n = 10–12 at 13 wk in B and C. The weight change differed between the two groups (P < 0.05) only when the selenium-deficient diet was fed. Brain selenium concentration differed (P < 0.05) at 7 and 13 wk. Brain glutathione peroxidase activity differed (P < 0.05) at all time points.
mice diverged when they were subsequently fed the selenium-deficient diet for 3 wk (Fig. 3B). After reconstitution of the 1.0 mg/kg diet, there was no “catch up” in the Sepp \(^{-/-}\) brain selenium concentration. Although other explanations for this are possible, feeding the selenium-deficient diet might have irreversibly damaged some of the cells in the brain that take up selenium. A systematic neuropathological evaluation is being carried out in affected mice to seek sites of injury to explain the clinical and biochemical findings.

A search of the literature revealed one report of neurological dysfunction in selenium-deficient rodents (11). Mice raised through 3 generations of selenium deficiency exhibited hind leg crossing when lifted by their tails. This was apparently the only nervous system abnormality that was observed and brain selenium concentrations were not reported. Thus, simple selenium deficiency of the brain, although extremely difficult to produce, might cause abnormalities similar to the mildest abnormalities reported here in Sepp \(^{-/-}\) mice.

The only function of selenoprotein P that has been identified with certainty is that it provides selenium to the brain and to the testis (3,4). Selenoprotein P is also expressed in the brain (12,13), and this indicates that it has a function within the brain as well. There is evidence that selenoprotein P serves to transport selenium to neuronal stem cells (14); thus, it is likely that it has a transport function among brain cells. Other functions such as oxidant defense are possible. Consideration of potential selenoprotein P functions is important in trying to determine the pathogenesis of the neurological injury seen in Sepp \(^{-/-}\) mice fed a low selenium diet. The injury might be simply a result of insufficient selenium in some parts of the brain or it might be caused by loss of an enzymatic function of selenoprotein P coupled with a low selenium supply to the brain. Further work will be necessary to evaluate these possibilities.

In conclusion, dysfunction of the brain occurs in young mice when selenoprotein P is absent and dietary selenium supply is low. A likely reason for the dysfunction would appear to be selenium deficiency in a critical compartment of the brain. These observations support the hypothesis that a major function of selenoprotein P is to transport selenium to the brain and, perhaps, within the brain.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**