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Effects of Prolonged Selenium Deficiency on Open Field Behavior and Morris Water Maze Performance in Mice

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WATANABE, C. AND H. SATOH. *Effects of prolonged selenium deficiency on open field behavior and Morris water maze performance in mice.* PHARMACOL BIOCHEM BEHAV **51**(4) 747-752, 1995.—Neurobehavioral effects of prolonged Se deficiency were evaluated using ICR mice. Dams were fed Se-deficient or Se-adequate diet starting at 4 weeks before conception through the suckling period. After weaning, offspring of both groups were given the same diet as their dams were given. The behavior of these offspring was evaluated with open field apparatus (OPF) and Morris water maze. In OPF, Se-deficient females exhibited less locomotor activity, more defecation, and less entry to the center square areas than did the control females. No such difference was found in males. In the Morris maze, Se-deficient females showed slight but significant impairment during the initial phase of the trials. The behavioral changes in OPF and the maze might have been due to an altered reactivity to a novel environment, although this possibility needs further confirmation. The obtained data showed that the altered behavior was unlikely to be due to the changes in thyroid hormones. Mechanism of these behavioral effects is discussed in relation to possible neurochemical changes induced by Se deficiency.

Selenium deficiency Open field behavior Morris water maze Brain selenium Glutathione peroxidase Mice

ESSENTIALITY of selenium (Se) in mammals as a constituent of glutathione peroxidase (GSH-Px) has been long established (12). The diversity of biochemical changes induced by Se deficiency [e.g., (24)] as well as the recent identification of several Se-containing proteins (7) suggested that there are many unknown biological functions of this element. Among them, the role of Se in brain or the neurobehavioral consequences of Se deficiency have been scarcely reported and are of particular interest.

There are two lines of evidence that suggested the importance of brain Se. First, handling of Se by the brain was much different from that by other organs, such as liver, and only the brain showed an enhanced retention of Se during Se deficiency (9,30). Because only a very small portion of brain Se binds to GSH-Px (27), other form(s) of Se must also be well preserved (7). Second, type I iodothyronine 5′-deiodinase (5′-DI) was identified as a selenoenzyme (8), the deficit of which in severe Se deficiency caused an imbalance of plasma thyroid hormones (5). Therefore, if the Se deficiency started in the early period of life, the hormonal imbalance might lead to permanent CNS dysfunction, as was once suspected in a human population (28). Despite these findings, the neurological or behavioral consequences of Se deficiency in this organ have rarely been examined. A recent report showed accelerated turnover of dopamine in the substantia nigra of Se-deficient rats (10).

Recently, we reported that Se-deficient preweaning mice, with decreased plasma T_3 and decreased brain GSH-Px activity, showed a retardation in the reflex/motor development and an altered preference of thermal environment (30). Because the Se-deficient status affected the neurobehavioral development of prenatal animals as such, a question arose whether the effects extend into the postweaning period.

The present study evaluated behavioral functions of Se-deficient mice in their postweaning period. The open field test and the Morris water maze were chosen as test items. The open field test is widely used as a test for emotional status and activity level [e.g., (2)] and is sensitive to many physiological

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and pharmacological perturbations. The Morris water maze, on the other hand, was developed as a test for spatial learning (23) and is responsive to many manipulations [e.g., (17)]. To assess the nutritional Se status, Se concentration, GSH-Px, and GSH-S-transferase (GSH-S-Tr) activities in the brain and plasma thyroid hormones were also determined.

METHOD

Diet and Animals

A Se-deficient diet based on Torula yeast was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). The Se-deficient diet contained less than 20 ng Se/g. The control diet was made by adding sodium selenite to the basal diet, making a final concentration of 400–440 ng Se/g. The mice were raised in a temperature-controlled room (23 ± 2°C) with a 12L : 12D photoperiod (0800–2000 h). The mice had free access to water. Parental ICR mice (7-week-old males, 4-week-old females) were purchased from Nippon SLC Co. Ltd. (Hamamatsu, Japan). The females were fed Se-deficient or control diets for more than 4 weeks and then mated with males fed on normal lab chow. After giving birth, the females nourished their own litter. The lactating dams were fed the same diet as they were fed before parturition. At 3 days after birth, litter size was reduced to six. At 19–21 days after birth, the litters were weaned and litter size was further reduced to four (two males and two females whenever possible). Details of the diet composition and raising procedure up to weaning are described elsewhere (30).

The weaning offspring were fed the same diet as their mothers were fed. Littermates of the same sex were housed in each cage. Offspring born to six Se-deficient dams and six control dams were used in the present study. Unless otherwise indicated, one male and one female mouse per litter were used. This study was carried out after permission from the Committee of Animal Experimentation, Tohoku University School of Medicine.

General Procedures

The open field test was conducted at 6 and 10 weeks of age, and the Morris water maze at 16 weeks of age. After finishing the open field test at 6 weeks, the mice used in the test were sacrificed for tissue analyses. Other groups of mice were used for the open field test at 10 weeks and the Morris water maze, and then were sacrificed at 18 weeks of age for the assays.

Open Field Test

The open field (OF) apparatus was made of Plexiglas. The floor was transparent, measured 50 × 50 cm, and was divided into 25 10 × 10-cm squares defined by black lines drawn on a sheet of paper that was placed under the floor. The floor was surrounded by a 20-cm-high, opaque black wall. The apparatus was illuminated by an 80-W fluorescent room light 2.5 m above the apparatus. Two water pumps in the room provided constant background noise. Each mouse was moved from its home cage to the center square of the OF. A box made of opaque black Plexiglas (10 × 10 × 10 cm) was then placed over the mouse. After 20 s, the box was gently removed, and the behavior of the mouse was observed for the following 2 min. The behavior was also recorded with a video recording device for counting the number of locomotor activities and for recording the position of the mouse. A locomotion was defined as a movement in which both the hind paws entered a new square. The 25 squares were classified as either peripheral (the 16 squares adjacent to the wall) or central (the nine remaining squares in the center). The scores were also classified according to whether the square entered was peripheral or central. Defecation (number of fecal boli) in the open field was also recorded. One trial per animal per day was done for 3 consecutive days. Between each trial the floor and the wall were cleaned with alcohol followed by wet cotton. All the trials were done between 1300 and 1700 h.

Morris Water Maze

The apparatus was based on the design of Grant et al. (17) with some modifications. A plastic water pool (115-cm diameter) was filled with water to a depth of 22 cm. The water was kept at room temperature (23 ± 2°C). The water was made opaque by adding powdered milk. In the hidden platform (HP) trials, a round, 8.6-cm-diameter platform made of white Plexiglas was placed 2 cm below the water surface in one of four positions (N, S, W, or E). For a given mouse, the platform was placed in the same position for each trial. The center of the platform was placed 24 cm from the radius. The north, west, south, and east of the water maze were identified by a video device, wooden boxes, a plane door, and animal steel racks, respectively, all of which were visible from the inside of the tank, which served as distant visual cues (23) for the mouse.

A mouse was released in the water at one of four randomly selected positions (NW, NE, SE, or SW) near the wall and facing the wall. The latency, defined as the time (in seconds) from the release of the mouse to the finding and climbing on the platform, was recorded. When a mouse could not find and climb on the platform within 180 s from the time of release, it was gently lifted out of the water by hand and placed on the platform for 30 s. In this case, a latency of 181 s was recorded. Each mouse was given four trials per day, which were separated by 1–1.5 h.

In the reversed platform (RP) trials, the protocol was the same as that in the HP trials, except that the location of the platform for a given mouse was moved to the opposite side in the maze (e.g., S to N). In the visible platform (VP) trials, the location of the platform was made visible by a 14-cm-high, green-colored cylinder mounted on the center of the platform. The test protocol was the same as that in the HP trials, except that the location of the platform was randomly altered from trial to trial. The VP trials were implemented to evaluate motivational and performance aspects of the water maze learning. This protocol was described in the original procedure (23), but relatively few studies have used it (13).

Each mouse went through HP trials from days 1 to 4, RP trials on day 5, and VP trials on day 6. Before the first HP trial, each mouse was placed in the pool without the platform for 3 min to examine their swimming ability and swimming pattern.

Se Determination

Tissue concentrations of Se were determined fluorometrically after washing the tissue samples with a mixture of nitrate and perchloric acid (31). The accuracy of the assay was assured by including a reference material, NIST #1577a. The determined values fell within the range of the certified value.

Measurement of Enzymes

Brain tissue was homogenized in 10 vol. of ice-cooled isotonic sucrose and centrifuged at 105,000 × *g* for 1 h at 4°C.

Activity of GSH-Px in the cytosol was determined by spectrophotometry (25) with *t*-butylhydroperoxide as the substrate at 37°C (19). Activity of GSH-S-Tr was also determined by spectrophotometry with 1-chloro-2,4-dinitrobenzene as the substrate (18). Protein was determined by Lowry's method (20).

Thyroid Hormones

Plasma thyroxin (T_4) and triiodothyronine (T_3) were determined with commercial enzyme immunoassay kits (Dainapak, Dainabot Japan Co. Ltd., Tokyo).

Statistics

Means of body weight, Se concentration, and enzyme activity were calculated and tested by Tukey's multiple comparison after one-way analysis of variance (ANOVA). The means of open field indices (total locomotion, proportion of center entry vs. total locomotion, and defecation) were calculated for each group. Because, as described in the Results section, effects of Se deficiency in males and females appeared different, data from each sex were analyzed separately. Thus, the data were first analyzed by two-way ANOVA where Se status and the trial were the factors. When a significant effect was obtained, a within-sex within-trial Tukey test was applied. Because the number of males used in the Morris maze was very small, the data were not subject to statistical tests. For females, the logarithms of the latency were used to calculate the daily group means, to which one-way ANOVA was applied. The level of significance in each of these analyses was $p < 0.05$ unless otherwise indicated.

RESULTS

Physical Growth, Chemical and Biochemical Measurements

At weaning, Se-deficient groups weighed less than control groups. The difference became smaller after 10 weeks of age, and disappeared at the end of the experimental period (18 weeks of age) (Fig. 1). The change in body weight paralleled the change in food consumption (data not shown).

The brain Se concentrations in the Se-deficient groups were reduced to about 60% of those of the controls (Fig. 2a), whereas the Se in the liver was almost depleted in the former groups (data not shown). The brain cytosolic GSH-Px activity was also reduced to one-half of the control groups (Fig. 2b).

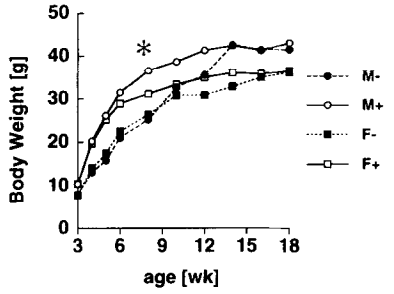

FIG. 1. Mean body weight of mice after weaning. For visual clarity, error bars have been omitted. Asterisk indicates that the Se-deficient groups are significantly different from the control groups of the same age ($p < 0.05$; Tukey's multiple range test). M−, M+, F−, and F+ stand for Se-deficient male, control male, Se-deficient female, and control female, respectively.

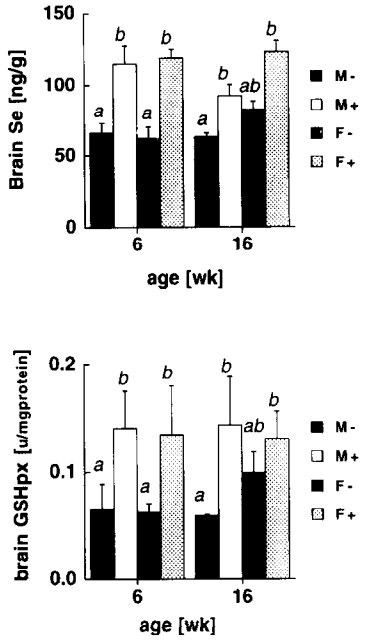

FIG. 2. Se concentrations (a) and GSH-Px activity (b) in the brain of mice. Mean and SD (vertical bars) are shown. Within the same age, groups with different letters are significantly different from each other by Tukey's multiple comparison ($p < 0.05$; Tukey's multiple comparison).

However, no compensational increase in the GSH-S-Tr activity was found in the brain cytosol (data not shown).

Se-deficient groups had slightly but significantly reduced levels of plasma T_3, probably due to the decreased activity of type I iodothyronine deiodinase, a selenoenzyme (8), whereas plasma T_4 levels were not affected (data not shown).

Open Field Test

Figure 3a shows the mean locomotion counts in the open field at 6 weeks of age. Because it appeared that the effect of Se status on this index was different between males and females, data from the two sexes were analyzed separately. A two-way ANOVA (Se and trial were the factors) for males revealed that neither factor was significant. The ANOVA for females revealed that both Se, $F(1, 29) = 21.1$, $p < 0.001$, and trial, $F(2, 29) = 4.84$, $p = 0.016$, were significant, whereas the interaction (Se × trial) was not. Within-trial Tukey tests showed that the differences between the control and the Se-deficient group were significant for females in trials 1 and 3; the difference was marginal ($p = 0.058$) in trial 2. No significant effect was shown on the percentage of central entry at this age (data not shown). The mean defecation counts are shown in Fig. 3b. The two-way ANOVA for males showed no significant effects of Se and of trial, whereas ANOVA for females showed a significant effect of Se, $F(1, 30) = 5.58$, $p = 0.025$. The Tukey tests applied for within-trial data did not show significant difference in any of the trials. Figure 4a shows the mean locomotion counts at 10 weeks of age. Neither Se nor trial was significant for males by two-way ANOVA. For females, the effect of Se was only marginal ($p = 0.066$). At this age, two-way ANOVA revealed a significant effect of Se on the percentage of central entry, $F(1, 24) = 9.54$ $p < 0.01$ (Fig. 4b). The within-trial Tukey test showed a significant

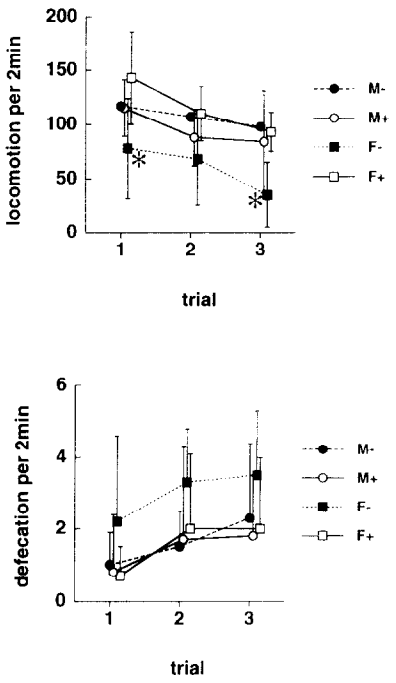

FIG. 3. Locomotion (a) and defecation (b) in an open field test at 6 weeks of age. Locomotion is expressed as the mean number of movements, and defecation is expressed as the mean number of fecal boli. Vertical bars indicate 1 SD. Asterisks indicate that within-trial difference between Se-deficient and the control groups of the same sex are statistically significant (Tukey's comparison; $p < 0.05$). $n = 6$ per group.

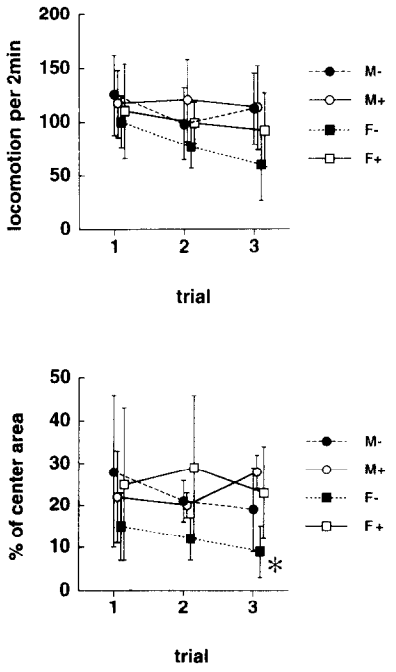

FIG. 4. Locomotion (a) and the proportion of movements made into central squares (b) in an open field test at 10 weeks of age. The meaning of vertical bars and asterisks is the same as in Fig. 3. $n = 4$–5 per group.

difference between Se-deficient and control females in the third trial ($p < 0.05$). No significant effect was found on the number of defecations at this age (data not shown).

Morris Water Maze

In the swimming test conducted before the first HP trial, all the mice were able to swim more than three minutes. The latency for climbing on the platform in the trials is shown in Fig. 5. One-way ANOVA, applied for the female groups, showed a significant effect of Se on the latency of the first day of the HP, $F(1, 6) = 10.3$, $p = 0.019$. No significant difference was found in the following trials (days 2–4). In addition, no significant group difference was found in RP and VP trials (days 5–6).

DISCUSSION

The present study showed that a long-term Se deficiency altered the behavior of female mice in the open-field apparatus and in the Morris water maze. The reduced Se concentration and the decreased GSH-Px activity in brain indicated that the Se-deficient mice were depleted of this element. These results, together with our previous report (30), demonstrated for the first time that long-term Se deficiency could exert behavioral effects.

In the open field test, the behaviors of females varied significantly with Se status; that is Se-deficient females showed less movement, more defecation (6 weeks), and less frequent entry to the center area (10 weeks) than did the control group. On the other hand, such a significant difference was not found with the males. Activity scores in the open field test are considered to reflect both exploratory behavior and the anxiety state in adult rats (1,29) and were increased by anxiolytics, such as chlordiazepoxide and oxazepam in rats (11) and in mice (13). Also, increased defecation (1,22) as well as less frequent entry into the central areas (32) were related to high emotionality in rodents. Thus, the behavioral patterns exhibited by the Se-deficient females might imply their hyperemotionality or high anxiety state. These psychological interpretations need to be evaluated more specifically either by employing other behavioral tests [such as the elevated plus-maze (26)] and/or by using pharmacological probes (e.g., challenging Se-deficient mice with anxiolytics).

In the Morris maze, the performance of the Se-deficient females was slightly but significantly impaired in the initial

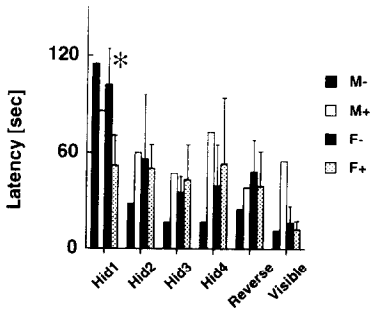

FIG. 5. Latency of each group in the Morris water maze. Hid, Reverse, and Visible stand for hidden platform, reversed platform, and visible platform tasks, respectively (see text). The meaning of vertical bars and asterisks is the same as in Fig. 3. $n = 4$ for females. No error bars are shown for males because of small group size.

phase of the HP trials. Because there was no group difference in the VP trials, the observed difference in the HP trials could not be explained by the differences in motivation, in motor performance [see (23)], or in visual acuity. The observed impairment, however, did not necessarily mean that the spatial learning of the Se-deficient group was impaired because no difference from the control was observed in the later HP trials and in the RP trials. It was reported that prenatal administration of oxazepam, a benzodiazepine, to mice induced a similar behavioral pattern (an impaired performance in the initial phase of the HP trials and normal performance in the subsequent HP trials and in the RP trials), which was considered to be more the result of a reduced adaptability to a novel environment than the result of an impaired spatial learning ability (14). Considering the aforementioned discussion of the results of the open field test, it is more likely that initial hampering in the Morris maze performance of the Se-deficient females was also due to altered reaction to a novel environment.

Little has been reported on the neurochemical or neuropathological effects of Se deficiency. Recently, increased turnover of dopamine in the substantia nigra was reported in Se-deficient rats, which was ascribed to decreased GSH-Px activity and subsequent enhanced oxidative stress (10). Because these rats, being deprived of Se for only 2 weeks and having a GSH-Px activity as much as 80% of Se-adequate rats (10), were in much milder Se deficiency than our mice were, a similar neurochemical change was likely to have occurred in our Se-deficient mice. Thus, modulation of the dopaminergic pathway might be responsible for the observed behavioral effects. In fact, behaviors in the open field test [e.g., (15)] and in the Morris water maze (4,15) were shown to be responsive to dopaminergic manipulations. Interestingly, neonatally administered dopamine, like the Se deficiency in our study, reduced open field locomotion of the female rats without affecting that of the males (16).

It is unlikely that the decreased plasma T_3 contributed to the observed behavioral alteration. Because T_4 deiodination in the brain is not catalyzed by the Se-containing type I 5′-DI (21), brain T_3 of the Se-deficient mice having a normal level of plasma T_4 would not decrease. Moreover, the change was much smaller than those that affected open field behavior (2,3,6) and spatial learning (3).

In summary, the behavioral patterns exhibited by the Se-deficient female mice suggested that these animals were different in their response to a novel environment. Although the mechanisms underlying the behavioral changes were not elucidated, enhanced oxidant stress due to the decreased GSH-Px might be responsible. Further researches employing different behavioral paradigms and neurochemical parameters are warranted. Also, the reason for the sex difference in the behavioral effects of Se deficiency remains to be elucidated.

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