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Brain and blood mercury and selenium after chronic and developmental exposure to methylmercury

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Abstract

Fish contain methylmercury and the potentially protective element, selenium. Blood and brain concentrations of these elements were determined in female rats after consuming AIN-93-based diets containing 0.06 or 0.6 ppm of selenium (Se) and drinking water containing 0, 0.5, or 5 ppm of mercury as methylmercury (MeHg) for 6 or 18 months. Brain and blood concentrations of mercury and selenium were also evaluated in neonates after gestational exposure. For adult rats in the high-Se, high-Hg condition, brain selenium content was 0.35 ppm and 1.8 ppm after 6 and 18 months, respectively, but for every other adult-onset condition, it was 0.1 ppm. Blood selenium varied less than two-fold despite a 10-fold difference in diet. After 6 months, mercury content in the brain showed a greater than 10-fold difference between the mercury groups, and interacted somewhat with dietary selenium. After 18 months, no mercury was detected in the brains of the 0.5 ppm groups, and their blood mercury also fell. For the 5.0 ppm groups, brain mercury increased slightly (low Se diet) or several-fold (high Se diet) over that seen at 6 months, and blood mercury also increased. Neonatal selenium concentrations were more labile than adults, and mercury in neonates was generally higher. All animals exposed to 5 ppm of mercury experienced a molar excess of mercury over selenium. Animals exposed to 0.5 ppm mercury showed a balance between mercury and selenium or a selenium excess, depending on the condition.

Keywords: Methylmercury; Selenium; Chronic exposure; Developmental exposure; Brain; Blood; Rat

1. Introduction

The major source of human exposure to methylmercury is the consumption of fish and seafood (Clarkson, 2002; Schober et al., 2003). These foods also represent a source of nutrients, including selenium (Chapman and Chan, 2000; Svensson et al., 1992), an important nutrient in its own right, and one that has been hypothesized to protect against methylmercury's neurotoxicity (Marsh et al., 1995). Selenium's physiological function in the nervous system is poorly understood, but the fact that the concentration of selenium and certain selenoproteins in the brain are aggressively defended in the face of severe depletion, suggests that this element plays a crucial role in neural function (Burk et al., 2005; Chen and Berry, 2003; Whanger, 2001).

Selenium and mercury bind with an exceptionally high affinity that sequesters mercury and possibly renders it biologically inert (Raymond and Ralston, 2004; Skerfving, 1978). This high affinity, which exceeds that of the mercury-sulfur bond by many orders of magnitude, may also reduce the bioavailability of selenium and decrease its incorporation into selenoenzymes. The selenium-mercury binding, which appears to be linked to inorganic mercury (Bjorkman et al., 1995), may be responsible for the complex effects on tissue levels when these elements are administered in combination. Increased dietary selenium results in an elevated deposition of mercury in key brain regions and delays the onset of neurological signs associated with high doses of methylmercury (Moller-Madsen and Danscher, 1991; Prohaska and Ganther, 1977). If ample selenium is also available, methylmercury administration causes transient increases in selenium uptake into the brain (Komsta-Szumska et al.) and its redistribution to mitochondria (Prohaska and Ganther, 1977).

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More poorly understood are the effects of methylmercury on selenium concentrations in the brain under conditions of normal dietary intake. Such dual exposure could lower the amount of biologically available selenium if, for example, the bound selenium continued to participate in homeostatic mechanisms regulating selenium concentration. In a developmental study involving dietary selenium levels that were low and high, but not excessively so, prenatal methylmercury exposure resulted in a substantially diminished concentration of selenium in the neonatal brain (Watanabe et al., 1999). This is remarkable because in adults it is very difficult to decrease brain concentrations of selenium by dietary manipulations alone. It has been claimed, for example, that after six generations of severe depletion, the concentration of selenium in the adult brain is still 60% of control even as that of liver, muscle, and blood were lowered to 1% of control (Behne et al., 2000; Chen and Berry, 2003).

Previous studies of brain selenium and mercury concentrations have mostly entailed short-term exposures to methylmercury concentrations so high that they could result in a large molar excess of mercury over selenium and severe neurological deficits. The present experiment was intended to examine the concentration of mercury and selenium in the brain and blood of rats under exposure regimens designed to model conditions of human exposure more closely than most previous investigations. In one regimen, chronic exposure to methylmercury was combined with a low- or high dietary selenium. Dietary exposure began at about 17–18 weeks of age, and after three weeks on the diet, methylmercury exposure began and continued for 6 or 18 months. These animals were mothers of the animals used for gestational exposure, the second regimen. Each exposure regimen, chronic and developmental, was conducted as a 2 (Se) × 3 (MeHg) factorial design that permits the examination of interactions between dietary selenium and methylmercury. Dietary concentrations of selenium were at the low and high end of recommended intakes. The low selenium concentration of 0.06 ppm in diet (0.76 μM/kg) is the lowest that can be obtained with a casein-based diet, a diet selected to facilitate comparisons with other studies from this laboratory involving polyunsaturated fatty acids. The high-concentration, 0.6 ppm, is at the high end of adequate but non-toxic levels (Reeves et al., 1993). Methylmercury concentrations, 0, 0.5, and 5 ppm, were similarly selected to span a range of exposures that might be characterized as low to moderate (Burbacher et al., 1990). Assuming 10 and 20 g of food and water intake, respectively, these concentrations result in 2.4 and 24 μg/kg/day (0.3 μmol/kg/day) of selenium and 0, 40, and 400 μg/kg/day (2 μmol/kg/day) of mercury. These, in turn, correspond to Hg:Se molar ratios spanning two orders of magnitude in exposed groups (Table 1).

2. Methods

2.1. Subjects

A total of 114, 16 week-old female Long-Evans rats were purchased from Harlan (Indianapolis, IN) and housed in conventional shoebox-cages with aspen bedding and open wire-bar tops in a temperature- and humidity-controlled, AAALAC-accredited colony room that was maintained on a 12-hour light-dark cycle (lights on at 7:00 a.m.). After a week of acclimation, the subjects were divided into six, weight-matched experimental groups, to form a 2 (dietary Se) by 3 (MeHg exposure) factorial design. Because the rats were to be used for breeding, they were originally assigned to groups such that there was over-representation of groups in which breeding difficulty was

Table 1
Experimental conditions and selected variables

Duration of Hg exposure	Se[a] (ppm)	Hg (ppm)	Hg intake[b] (μg/kg/day)	Se intake[b] (μg/kg/day)	Hg molar intake[b] (μmole/kg/day)	Se molar intake[b] (μmole/kg/day)	Hg:Se molar intake[b]	*N*	Hg:Se brain[c]	Brain: blood Hg ratio
6 months	0.06	0.0	0	2.4	–	0.03	–	5	–	–
	0.06	0.5	40	2.4	0.2	0.03	6.66	5	0.3 ± .06	0.13 ± .009
	0.06	5.0	400	2.4	2.0	0.03	66.60	6	13.3 ± 1.7	0.081 ± .009
	0.60	0.0	0	24.0	–	0.30	–	2	–	–
	0.60	0.5	40	24.0	0.2	0.30	0.66	5	0.5 ± 0.2	0.104 ± .009
	0.60	5.0	400	24.0	2.0	0.30	6.66	5	6.1 ± 0.6	0.103 ± .011
18 months	0.06	0.0	0	2.4	–	0.03	–	8	–	–
	0.06	0.5	40	2.4	0.2	0.03	6.66	7		
	0.06	5.0	400	2.4	2.0	0.03	66.60	3	11.8 ± 1.2	.084 ± .012
	0.60	0.0	0	24.0	–	0.30	–	5		
	0.60	0.5	40	24.0	0.2	0.30	0.66	7		
	0.60	5.0	400	24.0	2.0	0.30	6.66	5	3.7 ± 0.5	0.134 ± .009
Gestational[d]	0.06	0.0	0	2.4	–	0.03	–	9	–	–
	0.06	0.5	40	2.4	0.2	0.03	6.66	12	1.2 ± .06	0.251 ± .035
	0.06	5.0	400	2.4	2.0	0.03	66.60	13	18.7 ± .05	0.153 ± .022
	0.60	0.0	0	24.0	–	0.30	–	7	–	–
	0.60	0.5	40	24.0	0.2	0.30	0.66	10	0.45 ± .06	0.101 ± .025
	0.60	5.0	400	24.0	2.0	0.30	6.66	9	1.3 ± .06	0.158 ± .027

[a] Se exposure began 3 weeks before MeHg exposure.
[b] Estimates based on 20 mL/day fluid intake (Hg) and 10 mg/dayfood intake (Se).
[c] For gestational exposure, the geometric mean was used.
[d] Intakes based on maternal cosumption calculations.

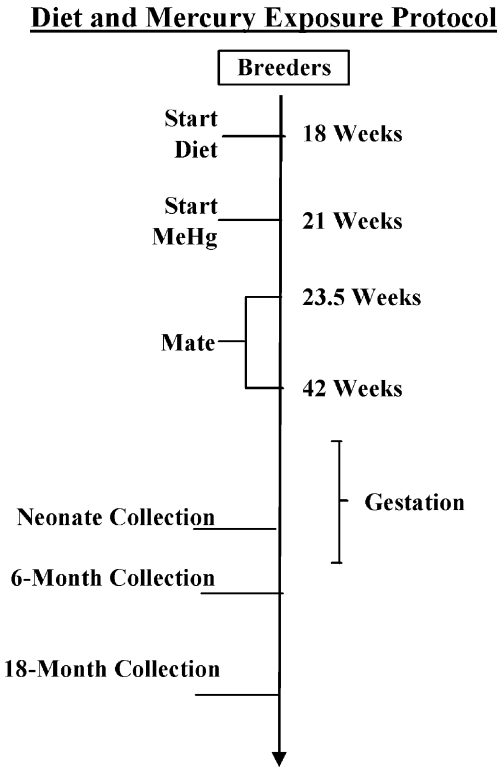

Fig. 1. Timeline showing exposure regimen. Breeders began their diet and exposure after arriving at the lab and continued for 6 or 18 months. Neonates experienced gestational exposure only.

expected (not shown). Specific sample sizes relevant to data analyses are shown in Table 1. Between 23.5 to 43 weeks of age, the breeder rats were mated with unexposed males and allowed to nurse their litters until weaning (Fig. 1). Prior to mating and after weaning, the rats were housed two/cage with a clear Plexiglas diagonal separator in the cage to permit individualized control over food and water provisions while maintaining appropriate space requirements. During breeding and while nursing, rats were housed individually in the same sized shoebox cages. Beginning at weaning, each breeder rat's body weight was reduced gradually to 250 g and feed was rationed to approximately 10 g/day so as to maintain this weight throughout behavioral studies (not described here) and to prevent their becoming obese.

Rats were inspected by veterinary and laboratory staff at least twice a week. Sentinel rats exposed to the same air and to bedding taken from selected rats used on the study were inspected semiannually for infectious diseases. All experiments were approved by the Auburn University Institutional Animal Care and Use Committee.

2.2. Selenium diet

At 18 weeks (125 days) of age, rats were placed on one of two diets, each based on the AIN-93 formula for laboratory rodents but customized for selenium concentration (Fig. 1, timeline). The "low selenium" diet contained selenium from casein only at a nominal concentration of 0.06 ppm. Actual selenium concentration ranged from 0.05 to, in one sample, 0.1 ppm. The "high selenium" diet was supplemented with sodium selenite to produce 0.6 ppm (analyses revealed actual concentrations between 0.6 and 0.9 ppm). During mating and pregnancy, the base diet was an AIN 93 growth diet containing 7% fat from soybean oil. Otherwise, the animals received a maintenance diet of an AIN 93 diet with 5.9% fat, as is appropriate for adult rodents. Both diets were obtained from Research Diets Inc (New Brunswick, NJ.). Dietary mercury was below the level of detection of 50 ppb.

2.3. Methylmercury exposure

At 20 weeks (145 days of age), after three weeks (20 days) on the custom selenium diets, each diet group was further divided into three methylmercury groups to create six experimental groups. Methylmercury exposures were 0, 0.5, or 5 ppm of mercury as methylmercuric chloride (Alfa Aesar, Ward Hill, MA) added to the drinking water. These concentrations produce approximately 0, 40, and 400 μg/kg/day of mercury, although these intakes can increase somewhat during gestation because of elevated fluid consumption during pregnancy (Newland and Reile, 1999). Fluid consumption reported in the earlier paper (Newland and Reile, 1999) was confirmed by taking periodic measurements of water intake. Drinking water was prepared from a stock solution containing 15 ppm of mercury as methylmercury. Every time a new dilution was created, actual mercury concentration was determined by atomic absorption and found to be within 10% of the target values.

2.4. Breeding

Breeding began at 23.5 weeks of age, after 3 weeks on MeHg. Rats were not exposed to MeHg through water from days 16 to 21 after giving birth, in order to prevent exposure to offspring, who could reach the bottle at those ages. After weaning, MeHg exposure via drinking water resumed and continued throughout life.

2.5. Tissue collection and elemental analysis

Animals were euthanized, and brain and blood were collected in adults at 46 weeks and 103 weeks (hereafter called one- and two-year-olds, respectively) of age, or 26 and 82 weeks of methylmercury exposure (hereafter called 6 and 18 months of exposure, respectively). For the neonates, whole brains from two littermates were used for mercury and selenium analyses. For the dams, whole brains, including cerebellum and midbrain, were cut sagittally and stored in polypropylene containers at −10 °C until they were shipped on ice to the Centre de Toxicologie/Institut National de Santé Publique du Québec (Sainte-Foy, QC, Canada) for analysis. Brains were collected after decapitation under anesthesia, which was induced by hypothermia for neonates and pentobarbital for adults. Brains were weighed and frozen until analysis. The right half of the dam's brain was used for selenium and total mercury analysis and the left hemisphere for inorganic mercury. Blood was collected from the axillary vein for neonates and from a cardiac puncture for dams, stored in EDTA vials,

weighed and frozen until analysis. In order to have enough blood from the neonates for analyses, blood from two littermates was combined and treated as a single sample.

Mercury and selenium content of blood and brain were analyzed using cold-vapor atomic absorption spectrometry (CVAAS) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. The analytical lab is accredited under ISO 17025 and participates in numerous external quality assessment schemes, including the U.S. CDC, the German Society of Occ. Medicine and Env. Medicine and the Centre de toxicologie's PCI scheme. The limits of detection were 50 ppb for total brain mercury, 200 ppb brain inorganic mercury, and 0.4 ppb for organic and inorganic mercury in blood. All tissues contained detectable levels of selenium.

2.6. Data analysis and statistics

Tissue content was analyzed as ppm and these values were converted to molar content (μM/kg) by multiplying by 5 for mercury and by 12.7 for selenium. Separate two-way analyses of variance were conducted for each analysis. Analyses included tissue content of total and inorganic mercury, selenium, and their molar ratios.

3. Results

3.1. Six-month exposure

There was an interaction between mercury and selenium on selenium concentration in the brain [$F(2,22) = 9$, $P = 0.002$]. Brain concentration of selenium was about 0.1 ppm (1.5 μM) for all exposure groups except those exposed to the high selenium diet and 5 ppm of mercury in drinking water; in that group, chronic exposure to MeHg resulted in a three- to four-fold elevation in brain selenium (Fig. 2).

There was a main effect of dietary selenium on selenium [$F(2,22) = 38$, $P < 0.001$], but not mercury [$F(1,16) = 2$, $P = 0.218$], concentration in the blood. There was a main effect

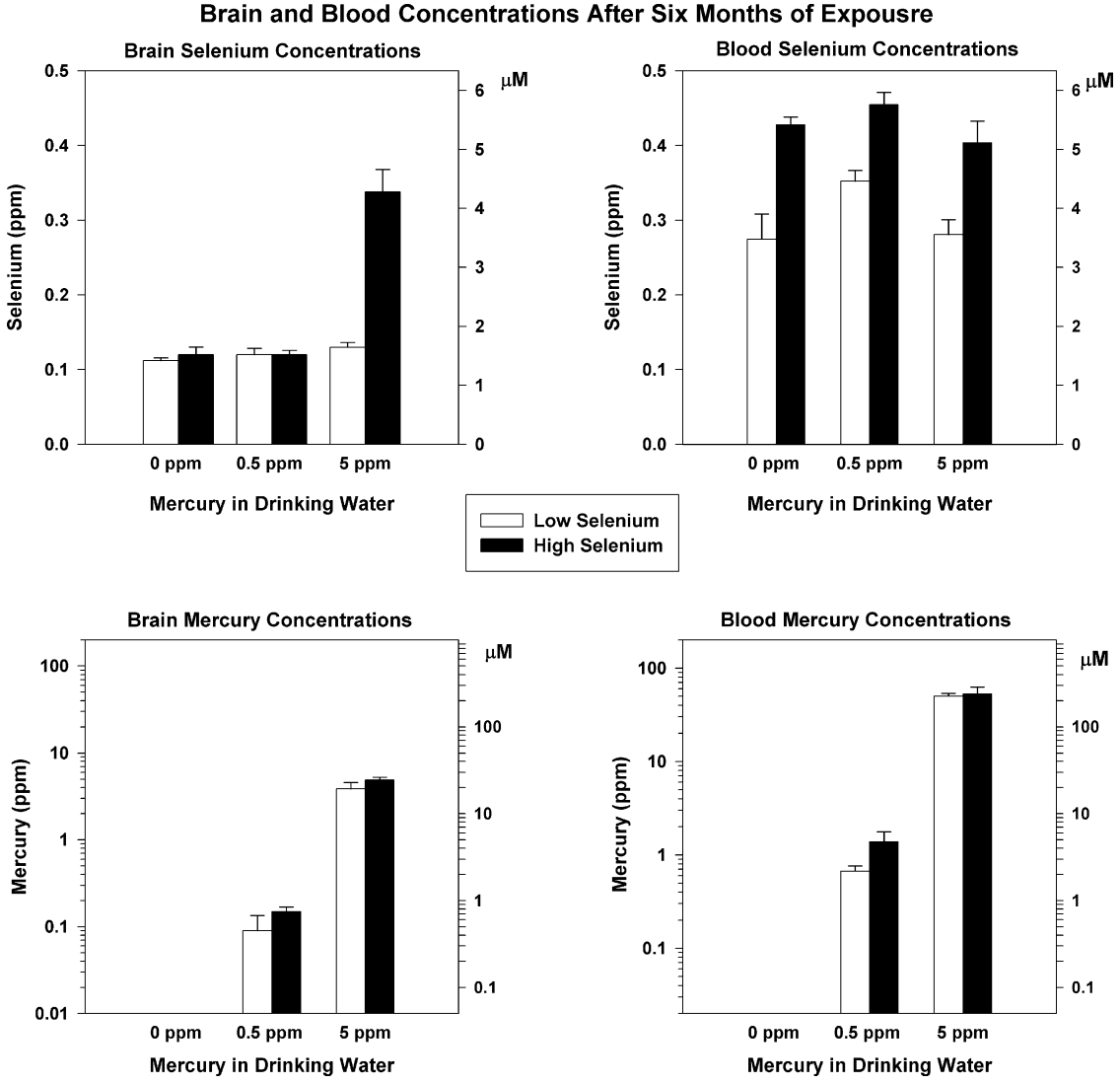

Fig. 2. Tissue levels of selenium (top) and mercury (bottom) in brain (left) and blood (right) for low (open bars) and high (filled bars) selenium groups after 6 months of methylmercury exposure. Left axes show levels as ppm and right axes as micromolar.

of mercury exposure on mercury content in brain [$F(1,17) = 81$, $P < 0.001$] and blood [$F(1,16) = 412$, $P < 0.001$]. There was no effect of selenium [$F(1,17) = 0.21$, $P = 0.651$] on mercury content in the brain, and no interaction on mercury content in the brain [$F(1,17) = 0.12$, $P = 0.736$] or blood [$F(1,16) = 2.8$, $P = 0.111$].

3.2. Eighteen-month exposure

There was a Hg × Se interaction [$F(1,29) = 18$, $P < 0.001$] on brain selenium. All exposure groups had about 0.1 ppm (1.5 μM) selenium in the brain except those exposed to high selenium and high mercury; the selenium content in the brain of these animals reached 2 ppm (25 μmolar) Fig. 3.

There was a Hg × Se interaction [$F(2,29) = 4$, $P = 0.031$] and a main effect of Se on blood selenium [$F(1,29) = 63$, $P < 0.001$]. Blood selenium concentration in the low selenium group was about 2/3 that of the high selenium group (0.31 versus 0.45 ppm, averaged across all MeHg groups). The interaction is due to the 5 ppm exposure groups: those on the low selenium diet had less blood mercury (0.26 ± 0.03) ppm than controls (0.33 ± 0.02) while those on the high selenium diet had slightly more (0.46 ppm ± 0.02) selenium than controls (0.41 ± 0.02).

There was an interaction between Se and Hg on brain mercury concentration after 18 months of exposure. There was no detectable mercury in the brains of rats exposed to 0 or 0.5 ppm of mercury. For those exposed to 5 ppm and low selenium, brain mercury concentrations were close to those seen after 6 months of exposure, but for those exposed to 5 ppm and high selenium, brain concentrations approximately doubled ([$F(1,18) = 7.0$, $P = 0.017$] for the Hg × Se interaction, ANOVA conducted on exposed rats, only).

There was a main effect of mercury [$F(1,18) = 127$, $P < 0.001$] on blood mercury after 18 months of exposure. There was an interaction between MeHg exposure and selenium [$F(1,18) = 5$, $P = 0.043$] on blood concentration of mercury. For the rats exposed to 5 ppm Hg, high dietary selenium increased blood mercury, but for rats exposed to 0.5 ppm, high dietary selenium decreased blood mercury.

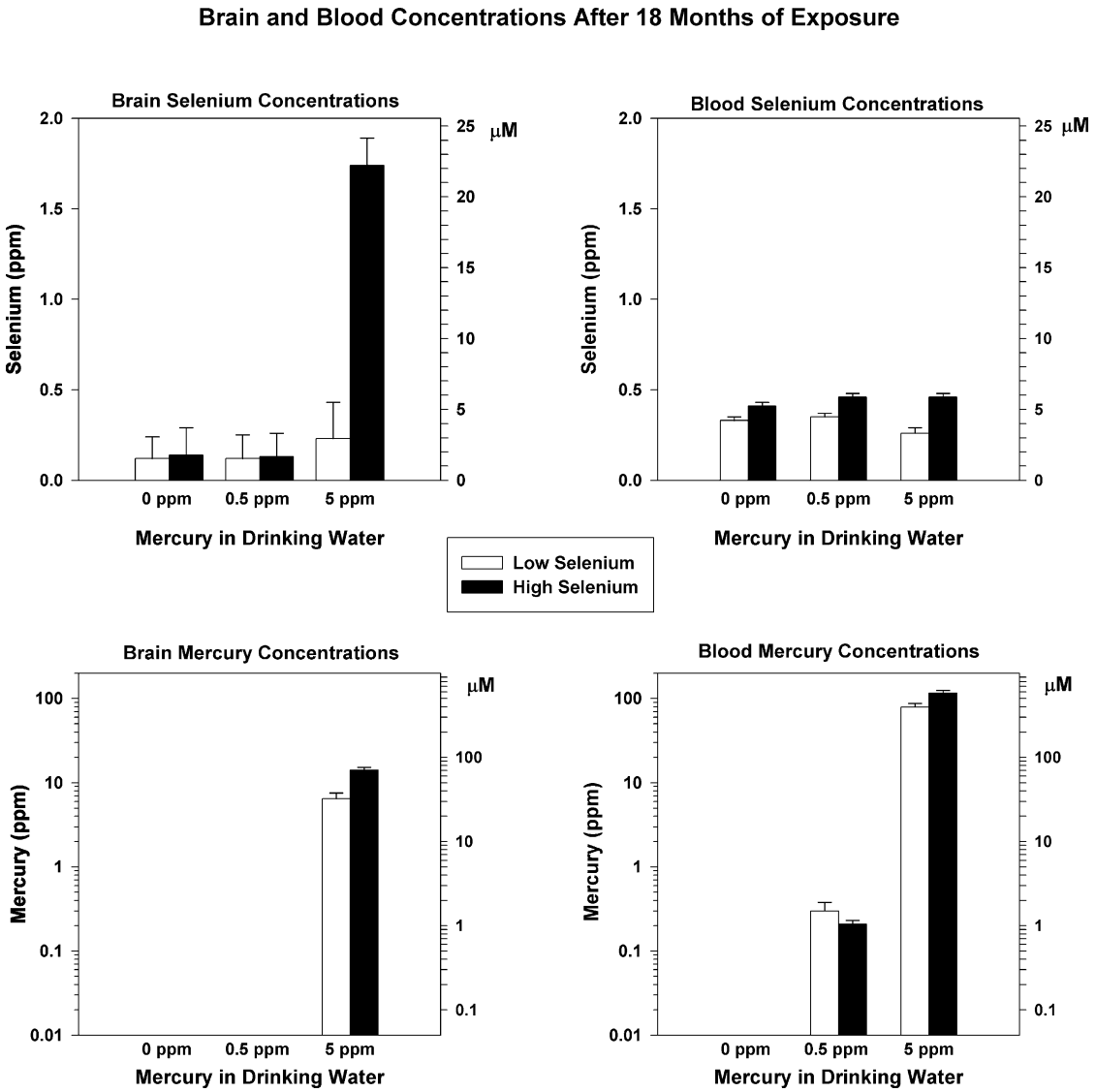

Fig. 3. Tissue levels of selenium and mercury after 18 months of exposure. Details as in Fig. 2. There was no detectable mercury in the brains of exposed to 0.5 ppm of mercury animals after 18 months of exposure.

3.3. Gestational exposure

There was a main effect of mercury [$F(2,54) = 21$, $P < 0.001$] and of selenium [$F(1,54) = 13$, $P = 0.001$] but no interaction on selenium concentration in the brain. There was a main effect of mercury [$F(2,49) = 19$, $P < 0.001$] and of selenium [$F(1,49) = 26$, $P < 0.001$], but no interaction on the concentration of selenium in the blood of neonates Fig. 4.

There was a main effect of mercury on mercury concentration in the brain [$F(1,38) = 98$, $P < 0.001$] and blood Hg [$F(1,25) = 20$, $P < 0.001$] of neonates but no effect of selenium and no interactions (Ps > 0.1).

3.4. Brain: blood mercury ratios

Statistical analyses were conducted on the blood:brain ratio because the variability across groups was more evenly distributed for this dependent variable than for its inverse. At 6 months of exposure, there was a Hg × Se interaction in blood:brain ratio [$F(1,16) = 6$, $P = 0.02$]. For the high selenium rats, this ratio was 10 in both exposure groups; while for the low selenium rats, the ratio was 7.8 and 12.4 in the 0.5 and 5 ppm exposure groups, respectively. At 18 months of exposure, these ratios in the 5 ppm group were the same as in the 1 year-olds, but there was no brain mercury detectable in the 0.5 ppm groups, so this ratio could not be determined at 18 months of exposure.

There was a Hg × Se interaction in blood:brain ratio [$F(1,25) = 7.2$, $P = 0.013$] for the neonates. For the high selenium animals, this ratio was 11.9 and 6.85 in the 0.5 and 5 ppm groups, respectively. For the low selenium animals, this ratio was 4.81 and 7.35 in these groups, respectively. Thus, the ratio was nearly the same for both groups of 5 ppm rats but was influenced by diet in the 0.5 ppm groups.

3.5. Effect of exposure duration

There was an interaction between exposure duration (6 versus 18 months of Hg exposure), mercury and selenium on selenium [$F(2,51) = 9.587$, $P < 0.001$] concentration in the

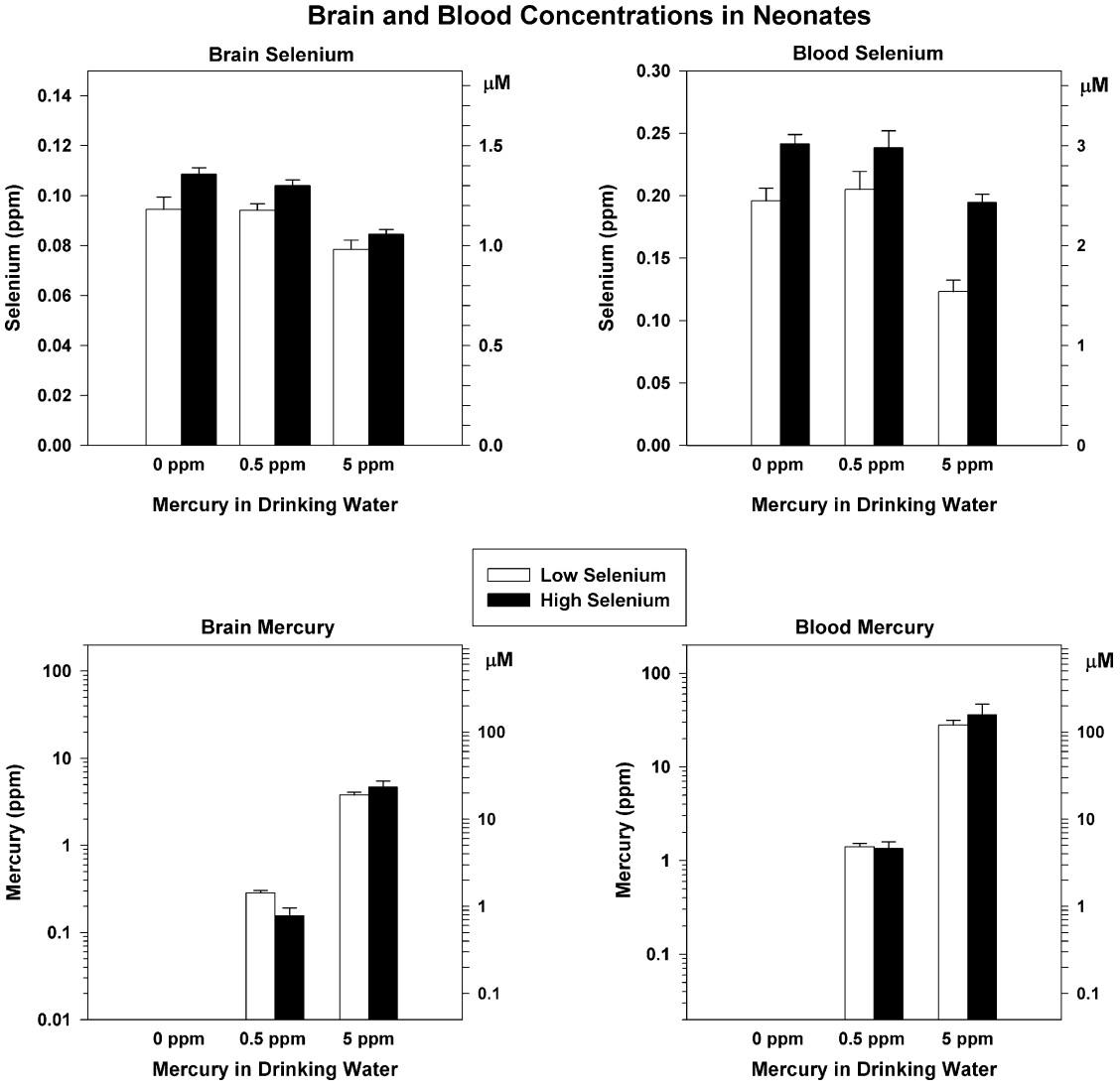

Fig. 4. Tissue levels of selenium and mercury after gestational exposure. Details as in Fig. 2.

brain. All exposure groups had about 0.1 ppm (1.5 μM) selenium in the brain at 6 and 18 months, except those exposed to high selenium and high mercury; the selenium content in the brain of these animals increased from 0.35 ppm (4.4 μmolar) at 6 months to 1.8 ppm (22.3 μmolar) at 18 months.

There was an interaction among exposure duration, mercury and selenium [$F(1,35) = 5.833$, $P = 0.021$] on mercury concentrations in the brain. This interaction was due to the rats that were exposed to 5 ppm of MeHg. In these, there was an increase in brain mercury from 5 ppm (25 μmolar) at 6 months to 7 ppm

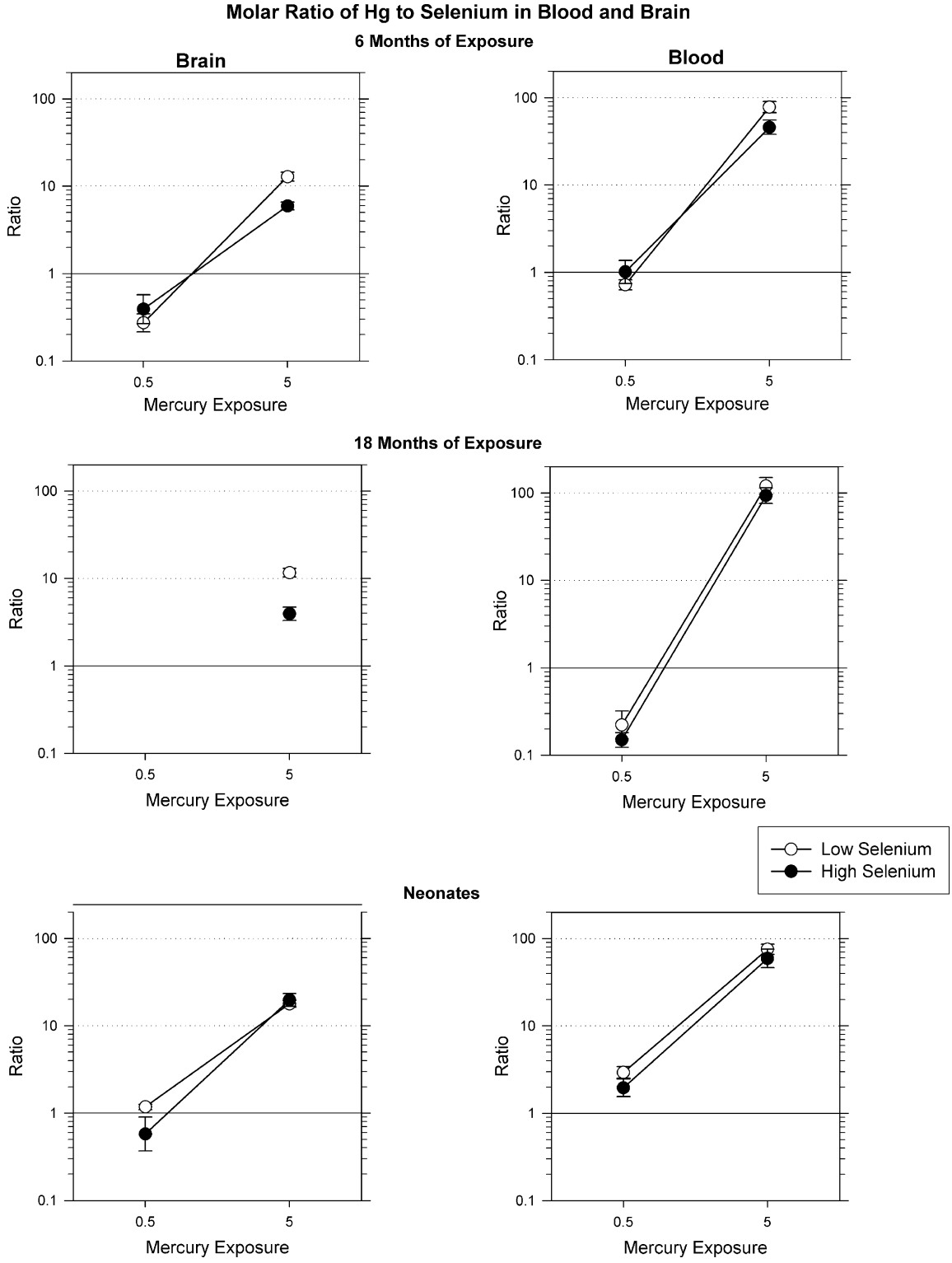

Fig. 5. Molar ratios of mercury to selenium. Where a value is missing, it is because there was no detectable mercury.

(35 μmolar) and 15 ppm (75 μmolar) for low and high selenium rats, respectively.

There was an interaction between duration of exposure and mercury [$F(1,34) = 18.97$, $P < 0.001$] on the concentration of mercury in the blood. Blood Hg concentrations decreased for the low mercury groups and slightly increased for the high mercury groups. Duration of Hg exposure had no effect on blood selenium concentrations (Ps > 0.1).

3.6. Molar ratios

At 6 months of exposure, there was a main effect of mercury on the Hg:Se ratio in the brain [$F(1,17) = 79$, $P < 0.001$] and in blood [$F(1,16) = 66$, $P < 0.001$] and an interaction between Hg and Se on this ratio in the brain [$F(1,17) = 13$, $P = 0.002$] and blood [$F(1,16) = 5$, $P = 0.043$] Fig. 5.

At 18 months of exposure, there was a main effect of mercury on the Hg:Se ratio in the brain [$F(1,18) = 347$, $P < 0.001$] and in blood [$F(1,18) = 105$, $P < 0.001$]. Because there was no detectable mercury in the brains of the low selenium, low Hg rats, the Hg:Se ratios could not be determined, but for the 5.0 ppm exposure groups, this ratio is significantly higher in the brains of animals on the low selenium diet than in those on the high selenium diet.

For the neonates, there was a significant effect of mercury on the Hg:Se ratio in the blood [$F(1,25) = 242$, $P < 0.001$], but there was no main effect of selenium and no mercury by selenium interactions (Ps > 0.1). In the neonatal brains, there was a main effect of mercury [$F(1,38) = 473$, $P < 0.001$] and selenium [$F(1,38) = 9$, $P < 0.01$], but there was also an interaction [$F(1,38) = 12$, $P < 0.001$]. The interaction occurred because for the 0.5 ppm-exposed neonates, the Hg:Se ratio was higher in the low-selenium group than in the high-selenium group (0.45 versus 1.2).

3.7. Inorganic mercury (Fig. 6)

At 6 and 18 months of exposure, there was no detectable inorganic mercury in the brains of rats exposed to 0, 0.5 ppm of mercury, or for those in the low selenium, 5.0 ppm group. For those animals exposed to a diet high in Se and 5 ppm of mercury, inorganic brain Hg increased about 10-fold between 6 and 18 months [$F(1,8) = 15.902$, $P = 0.004$]. For these animals, there was a nearly five-fold molar excess of selenium to inorganic mercury at 6 months of age, but this value got closer to 1.0 after 18 months of exposure.

For the 5 ppm Hg groups, there was an interaction between duration of exposure and selenium [$F(1,34) = 4.5$, $P = 0.04$] on inorganic mercury in the blood. At 6 months of exposure, blood inorganic mercury was about 8.5 ppm (42.5 μM) for both diet groups, but after 18 months of exposure, this value increased to 9.9 ppm (49.5 μM) and 18.6 ppm (93 μM) for the low and high selenium diet groups, respectively. The molar ratio of inorganic Hg:Se in the blood was 12.6 and 8.3 at 6 months of exposure and increased to 16.5 and 16.1 for the low and high selenium diet groups, respectively.

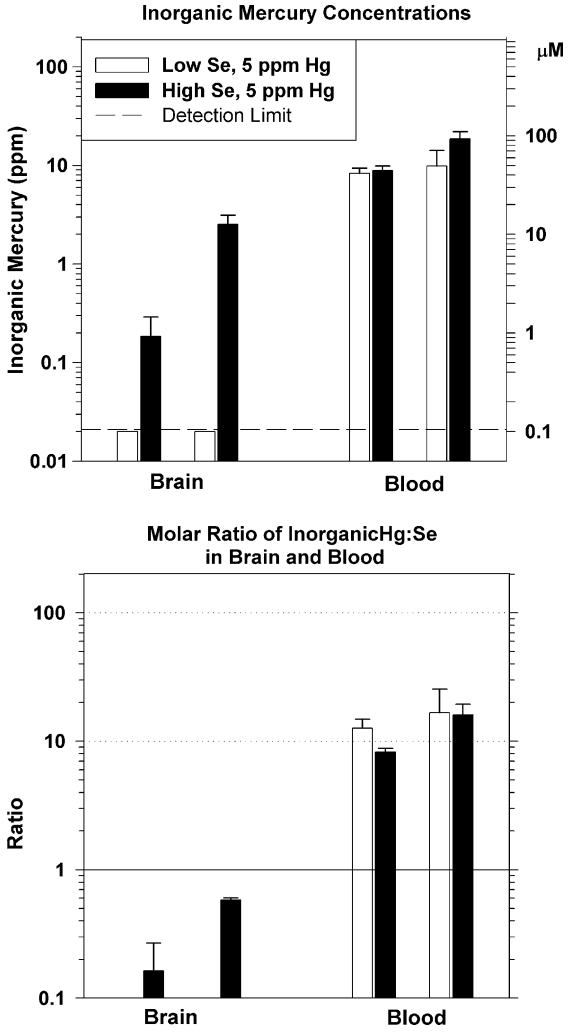

Fig. 6. Tissue levels of inorganic mercury (top) and molar ratios of inorganic mercury to selenium (bottom) for the low selenium, 5 ppm Hg group (open bars) and high selenium, 5 ppm Hg group (closed bars) after 6 and 18 months of exposure. The dashed line in the top figure indicates the detection limit of 200 ppb. Bars below this had no detectable inorganic mercury.

For the 0.5 ppm Hg groups (not shown in Fig. 6), there was a duration-of-exposure effect [$F(1,20) = 18.6$, $P < 0.001$] on blood inorganic mercury, which decreased from 0.1 ± 0.03 ppm and 0.18 ± 0.03 ppm at 6 months of exposure to 0.04 ± 0.02 ppm and 0.03 ± 0.02 ppm at 18 months of exposure for the low and high dietary selenium groups, respectively. The inorganic Hg:Se blood ratio for the 0.5 ppm groups (not shown in Fig. 6) was 0.11 ± 0.02 and 0.13 ± 0.02 at 6 months of exposure and decreased to 0.04 ± 0.017 and 0.028 ± 0.02 at 18 months of exposure for the low and high selenium groups, respectively.

4. Discussion

Numerous authors have noted that that selenium can delay the onset of some signs of methylmercury's neurotoxicity or otherwise confer some protection (Magos, 1991; Raymond and Ralston, 2004; Skerfving, 1978; Watanabe, 2002; Whanger, 1992). Joint administration of selenium and methylmercury

increases the deposition of methylmercury in nerve cell bodies in selected regions of the brain, including the cerebral and cerebellar cortex, and reduces or delays methylmercury's neurotoxicity (Moller-Madsen and Danscher, 1991). Quantitative information from long-term exposure protocols is lacking, but some short-term effects have been identified. Selenium induces a transient increase in mercury uptake into the brain (Skerfving, 1978; Watanabe, 2002). Acute administration of high doses of methylmercury decreases the uptake of selenium in the adult brain (Komsta-Szumska et al., 1983) and promotes its redistribution from cytosol into mitochondria (Prohaska and Ganther, 1977), at least transiently.

The present studies were designed to examine selenium and methylmercury levels in brain and blood under steady-state conditions designed to model those of human exposure. Rats consumed 0, 0.5, or 5 ppm of methylmercury chronically, concentrations that produce approximately 0, 40, or 400 μg/kg/day of mercury intake and low to moderate concentrations of mercury in the brain (Day et al., 2005). This intake overcomes the high mercury binding to hemoglobin seen in rats, so it may be higher than intakes that would be used with other species (Magos, 1987; Newland and Reile, 1999). The 5 ppm concentration produced neurological signs characteristic of methylmercury exposure after about 9 months of exposure (Day et al., 2005). The severity of signs, and number of animal showing them, increased over the subsequent year. Selenium can delay this onset somewhat ((Reed and Newland, 2005); paper in preparation). By comparison, the 0.5 ppm concentration never produced neurological signs, even after 2 years of exposure. Thus, the water concentrations of 0.5 and 5 ppm straddle a dose that does not produce classic neurological indicators of chronic methylmercury exposure and a dose that produces these, but gradually and after extended exposure.

4.1. Selenium

Several authors have noted that selenium's roles in the brain, which remains poorly understood, are likely to be important ones since brain content is so difficult to perturb in adults through dietary manipulations (Burk and Hill, 1993; Burk et al, 2005; Chen and Berry, 2003; Rayman, 2000; Whanger, 2001). Much brain selenium is found in selenoproteins, including the antioxidant, GSH-Px, (Burk and Hill, 1993), and selenoproteins P and W, whose functions have not yet been identified. These selenoproteins are more sensitive than total selenium to dietary content, but they are still more stable in the brain than they are in other organs (Burk and Hill, 1993; Hill and Burk, 1997; Hill et al., 2001; Whanger, 2001). Nevertheless, they do appear to play a role in selenium homeostasis in the nervous system since knocking out selenoprotein P can reduce total content of brain selenium (Burk and Hill, 1993).

In the present study, selenium concentration in the adult brain was about 0.1 ppm, 1.5 μM, in all but one condition, and under no condition did it fall below this level. When there was ample dietary selenium and exposure to 5.0 ppm of mercury, the selenium concentrations in the brain increased three- to four-fold after 6 months of methylmercury exposure and almost 20-fold after 18 months of methylmercury exposure. In contrast, blood selenium was unaffected by MeHg exposure, but it did reflect dietary Se exposure. Even so, the discrepancy in blood selenium concentration was much smaller than that in the diets. A 10-fold difference in dietary selenium resulted in a less than two-fold difference in blood selenium. Blood selenium in these groups did not change with age or duration of exposure.

4.2. Selenium content in neonates

Selenium in the brain and blood of neonates was more labile than in adults, confirming earlier reports investigating development exposure to methylmercury and selenium (Watanabe, 2001; Watanabe et al., 1999). The brain generally showed less variation in selenium content than did blood. In blood, the methylmercury and selenium combined multiplicatively, i.e., as a statistical interaction, but in the brain, the effects of the two interventions combined additively. The effect of each treatment, increased MeHg or reduced dietary Se, resulted in no more than a 20% decrease in selenium, so again, the magnitude of the effect was smaller than the ten-fold ratio in selenium content of the two diets, but it was consistent and dose-related.

4.3. Mercury concentration

After 6 months of methylmercury exposure, a 10-fold increase in drinking-water mercury, from 0.5 to 5 ppm, resulted in an approximately 50-fold increase, from about 0.1 to 5 ppm, in brain mercury. This is consistent with other reports of nonlinearity in the relationship between methylmercury intake and brain content (Day et al., 2005; Newland and Reile, 1999). A slight increase in brain mercury associated with the high selenium diet is discussed below.

After 18 months on mercury, a dramatic distinction between the two methylmercury-exposure groups appeared. There was no longer detectable mercury in the brains of animals exposed chronically to 0.5 ppm of mercury, regardless of diet. For the rats exposed to 5 ppm, the change in brain concentration was depended upon the diet. For rats on the low-selenium diet, brain mercury was similar at both 6 and 18 months, but for rats on the high-selenium diet, brain mercury approximately doubled over its content at 6 months. Thus, mercury content declined in the low Hg exposure group and either remained stable or increased in the high Hg exposure group, depending on dietary selenium.

Blood mercury concentration showed a similar pattern to that of brain mercury. Together, these suggest that at low exposure levels (0.5 ppm) there is some tolerance to oral exposure to methylmercury and that this involves a peripheral, i.e., non-neural, mechanism. This raises a provocative possibility that differential sensitivity associated with developmental and adult-onset exposure is due, at least partly, to toxicokinetic mechanisms.

After 18 months, blood mercury increased in the 5 ppm animals, and this increase was larger in those on the high selenium diet. Blood mercury concentration in the 0.5 ppm groups, 0.2–0.4 ppm, is consistent with the failure to detect mercury in the brains. Brain mercury concentration tends to be

about 10% of blood mercury, so brain mercury would be below the 0.05 ppm level of detectability.

For neonates exposed to 0.5 ppm, the brain mercury was higher than it was for those on chronic exposure to this concentration. A diet rich in selenium decreased mercury content in the neonatal brain at this low level of exposure. At the higher exposure level, there was no effect of diet.

4.4. Brain: blood ratios of mercury

The inverse of this number was used for statistical analyses, but the brain:blood ratio will be discussed here to be consistent with the broader literature and to emphasize brain content. For the high selenium adults, brain mercury was about 10% that of blood mercury, regardless of mercury exposure. For the low-selenium adults, the brain:blood ratio was higher (13%) in the 0.5 ppm groups than in the 5 ppm group (8%). Thus, with a near deficiency of selenium, the brain:blood ratio was sensitive to dose and was higher in the low methylmercury exposure condition, but with a high selenium diet, the brain:blood ratio was independent of intake. This ratio could not be evaluated at 18 months of exposure for either 0.5 ppm condition because brain mercury was not detectable, but for the rats exposed chronically to 5 ppm, it was the same after 18 months of exposure as it was after 6 months.

Thus, a diet rich in selenium stabilized the ratio between brain and blood mercury after chronic exposure. This interpretation differs somewhat from others by drawing attention to the stabilizing influence of selenium associated with chronic, low-level exposure. Other reports have emphasized the transient deposition of selenium and mercury in the brain after acute, high-level exposure.

Brain:blood ratios in neonates showed a different pattern than that seen in the adults. In neonates, as compared with adults, more variability and greater sensitivity to selenium content was seen in the rats exposed to 0.5 ppm of mercury. For neonates whose mothers were on a high-selenium diet, the brain:blood ratio was 0.10 and 0.16 in the 0.5 and 5 ppm groups, respectively. For those on the low selenium diet, these numbers were 0.25 and 0.15 in the 0.5 and 5 ppm groups, respectively. By comparison, when a chow diet was used, but otherwise the exposure regimen was similar, the brain:blood ratios were 0.13 to 0.16, respectively, for the two exposure groups (Newland and Reile, 1999).

4.5. Molar content of selenium and mercury

The effects discussed so far are rather complex, but the joint effects of mercury and selenium on neurotoxicity might be reducible, at least as a first approximation, to the question of whether there is a molar excess of mercury or selenium in the brain (Raymond and Ralston, 2004; Skerfving, 1978). To examine this possibility, the concentrations of mercury and selenium were converted to molar units so as to compare the number of selenium and mercury atoms in whole brain. With regard to neurological effects, the specific observations to be accounted for are: (1) chronic exposure to 5 ppm of methylmercury produces overt neurological signs but chronic exposure to 0.5 ppm does not, even after 2 years of exposure (Day et al., 2005) and (2) a diet rich in selenium can delay the onset of neurological signs (Moller-Madsen and Danscher, 1991; Prohaska and Ganther, 1977). The role played by specific brain regions is not addressed here.

In view of reports that selenium content is difficult to perturb in the brain, it is noteworthy that selenium was elevated in the high-selenium, high-mercury condition. This could suggest that homeostatic mechanisms draw from a pool of excess selenium, if available, to replace unavailable, mercury-bound selenium. Such a process would elevate selenium in a high-mercury, high-selenium group as reported here, and implies that the target of this regulation is not raw selenium content, but biologically active selenium, such as that incorporated into selenoproteins.

Mercury exposure coupled with a diet that is marginal in selenium content would, therefore, pose a significant neurological challenge if low selenium intake prevents the replacement of mercury-bound selenium. In such a situation, what appears to be adequate tissue levels of selenium levels could mask a deficit in the selenium that is actually bioavailable. Such a situation might be present in the animals in the high mercury (5 ppm) and high selenium condition. This was the only condition in which the brain content of selenium of adults was elevated, yet the increase was still insufficient to bind all the mercury, total or inorganic, present. The molar excess of mercury over selenium means that the potential still exists for unbound mercury to have neurotoxic effects, and simultaneously, for a deficit of bioavailable selenium if much of the brain selenium is bound to mercury. It can be noted that the conditions that produce a molar excess of mercury over selenium also result in the appearance of overt neurological signs, while conditions that produce molar excesses of selenium do not produce these signs.

For the animals exposed to 5 ppm of mercury, both brain mercury and selenium concentrations were sensitive to dietary selenium. This is qualitatively consistent with the notion that selenium-Hg binding elevates the concentration of these elements in the brain. The molar content of both elements was indeed higher for animals on the high selenium diet. Close examination of the values, however, reveals that selenium-mercury binding cannot account for all of the increase in brain mercury across the diet groups. At 6 months of exposure, brain mercury in the 5 ppm groups was about 20 and 25 μM (a 5 μM difference) in the low- and high-selenium groups, respectively, while brain selenium was 1.6–4.2 μM (a 2.6 μM difference) in these diet groups. Similarly, after 18 months of exposure, brain mercury in the 5 ppm Hg group was 32 and 70 μM (a 38 μM difference) while brain selenium was 3 and 23 μM (a 20 μM difference) in these diet groups. Quantitatively, the molar increase in mercury content associated with a 10-fold increase in dietary selenium was about twice that of the increase in selenium. Something in addition to selenium was responsible for the higher mercury content in the brains of animals on the high selenium diet.

An apparently reduced homeostatic control over selenium during early development is evident. Neonates in the 0.5 ppm-Hg groups had a slight molar excess of mercury when on the

low selenium diet and a molar excess of selenium when on the high selenium diet. This suggests that mercury may be bound and perhaps nontoxic in these groups, but it also raises the possibility of a functional selenium deficiency in the developing brain even at this low mercury exposure level. In the 5 ppm group, there was a 20-fold excess of mercury over selenium, nearly twice that seen in the adults.

The pattern seen in blood resembles that seen in brain, but the effects of mercury are more extreme. There was a 100-fold molar excess of mercury over selenium in the blood of rats on the 5.0 ppm diet, as compared with a 10-fold excess in the brains. It is possible that this molar excess reflects, not just selenium, but also the high mercury binding capacity of rat blood (Magos, 1987).

Overall, the molar ratios are highly informative. They indicate that the dosing regimen of 0.5 and 5.0 ppm spans an important threshold that is defined by whether there is a molar excess of mercury or selenium in the brain. At a 5 ppm exposure level, there was always a large molar excess of mercury over selenium in both brain and blood. Whether this is so for the 0.5 ppm group depends on whether exposure begins at adulthood or is developmental. If exposure begins at adulthood, then selenium atoms outnumber mercury atoms in whole brain, while if exposure is developmental only, these two elements are approximately in balance, and the content of mercury and selenium in the developing organism are especially labile. These results raise a provocative hypothesis that the vulnerability of the developmental period to methylmercury is due in part to toxicokinetic factors, in addition to the generally recognized vulnerability of the developing brain.

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