Hypoalgesia and recovery in methylmercury-exposed rats

Yo Shinoda¹, Yuta Yamada¹, Eiko Yoshida², Tsutomu Takahashi¹, Yayoi Tsuneoka¹, Komyo Eto³, Toshiyuki Kaji² and Yasuyuki Fujiwara¹

¹Department of Environmental Health, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
²Department of Environmental Health, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
³Health and Nursing Facilities for the Aged, Jushindai, Shinwakai, 272 Ikurakitakata, Tamana, Kumamoto 865-0041, Japan

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ABSTRACT — Methylmercury (MeHg), the causal substrate in Minamata disease, can lead to severe and chronic neurological disorders. The main symptom of Minamata disease is sensory impairment in the four extremities; however, the sensitivity of individual sensory modalities to MeHg has not been investigated extensively. In the present study, we performed stimulus-response behavioral experiments in MeHg-exposed rats to compare the sensitivities to pain, heat, cold, and mechanical sensations. MeHg (6.7 mg/kg/day) was orally administered to 9-week-old Wistar rats for 5 days and discontinued for 2 days, then administered daily for another 5 days. The four behavioral experiments were performed daily on each rat from the beginning of MeHg treatment for 68 days. The pain sensation decreased significantly from day 11 onwards, but recovered to control levels on day 48. Other sensory modalities were not affected by MeHg exposure. These findings suggest that the pain sensation is the sensory modality most susceptible to MeHg toxicity and that this sensitivity is reversible following discontinuation of the exposure.

Key words: Methylmercury, Hypoalgesia, Sensory modality, Minamata disease

INTRODUCTION

Methylmercury (MeHg) is an organic mercury compound known to cause Minamata disease (McAlpine and Araki, 1958; Eto, 1997). One of the main symptoms of Minamata disease and other MeHg-exposed cases is a sensory disruption in the distal portions of the four extremities (Hunter et al., 1940; Hunter and Russell, 1954; Bakir et al., 1973; Harada, 1995; Takaoka et al., 2008, 2014); however, the effects of MeHg on sensitivity to sensory modalities such as pain, cold, heat, and mechanical sensations have not been fully investigated.

Clinical sensory studies have shown a decline of two-point discrimination (Ninomiya et al., 2005) and impaired tactile sensation (Takaoka et al., 2004) in Minamata disease patients, as well as reduced sensitivity to heat and pain sensations in Minamata disease patients (Takaoka et al., 2008) and MeHg-exposed cases (Takaoka et al., 2014). In rats, MeHg impairs the tactile-kinesthetic system (Elsner, 1991), reduces sensitivity to electrical shock (Wu et al., 1985), and reversibly decreases the nociceptive response (Chuu et al., 2007). In monkeys, MeHg elevates the threshold of vibration sensitivity (Rice and Gilbert, 1995). These findings confirm that several sensory modalities are critically dysregulated by MeHg, but to our knowledge, there are no comparative investigations of MeHg susceptibility in multiple sensory modalities.

In the present study, we performed stimulus-response behavioral experiments for several sensory modalities in MeHg-exposed rats and evaluated the susceptibility of each sensory modality to MeHg.

MATERIALS AND METHODS

Animals and MeHg administration

Animal breeding and MeHg administration were performed as described previously (Shinoda et al., 2019a). Eight-week-old male Wistar rats (240-305 g, Tokyo Laboratory Animals Science, Tokyo, Japan) were housed in cages under a 12/12-hr light-dark cycle, with
ad libitum access to water and food. MeHg administration began when the rats were 9 weeks old. Methylmercury chloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in MilliQ water (MilliporeSigma, Burlington, MA, USA) to a concentration of 2 mg/mL. The MeHg solution was administered orally using a gastric tube at a daily dose of 6.7 mg/kg for 5 days, followed by 2 days without MeHg and a further 5 days of MeHg dosing. Age-matched rats administered water were used as controls. All behavioral and health condition tests were performed daily from the first day of MeHg administration. All experimental protocols were evaluated and approved by the Regulations for Animal Research committee at Tokyo University of Pharmacy and Life Sciences. All efforts were made to minimize the number of animals used and their suffering.

Behavioral tests

General description

Forty two (20 control and 22 MeHg-exposed) rats were separated into two groups on the first day of MeHg administration. The first group (10 control and 11 MeHg rats) underwent the foot shock test and tail immersion test, and the second group (10 control and 11 MeHg rats) was assessed by the tail flick test and von Frey test. The tests were performed once a day from the first administration of MeHg for 68 days. One of the MeHg-exposed rats in the second group died during the experiment, so data from that rat were excluded.

Analysis of pain sensation

Pain sensation was assessed via the foot shock test. Rats were placed in a plastic box (34 cm wide × 26 cm deep × 28.5 cm high) with an electrifiable grid floor (O’Hara & Co., Ltd., Tokyo, Japan). The rats were exposed to electrical shock for 0.5 sec using a shock generator (SGA-2010, O’Hara) from 0.01 mA and the amplitude was increased until the rats exhibited a shock response. The intensity of the minimal response-inducing shock amplitude was recorded.

Analysis of cold sensation

Cold sensation was assessed using the tail immersion test. A beaker containing 1 L ice-cold water was placed in a box of crushed ice to maintain the temperature at 4°C. Each rat was held in the operator’s hand and handled until relaxation. Next, the terminal 5 cm of the tail were immersed in the water. The endpoint was characterized by a jerk of the tail. The time between the start of tail immersion and the jerk of the tail was measured.

Analysis of heat sensation

Heat sensation was assessed by the tail flick test. Each rat was held in the operator’s hand and handled until relaxation, then placed on a table. Next, hot air (75°C) from a heat gun (Goomand, Tokyo, Japan) clamped 8 cm from the table was blown against the middle of the tail. The time it took the animal to move its tail out of the hot air stream was recorded.

Analysis of mechanical sensation

Mechanical sensation was assessed by the von Frey test. von Frey filaments (North Coast Medical, Morgan Hill, CA, USA) ranging in force from 8 to 300 g were applied in ascending force, with each filament applied two to three times to the instep surface of the hind paws of hand-held relaxed rats. The lowest force that elicited nocifensive behaviors (including extended paw withdrawal and shaking of the paw) in two of three applications was considered and recorded as the paw-withdrawal threshold.

Hind-limb crossing test

The hind-limb crossing test was performed by scoring the position of the rat’s hind limbs when suspended by the tail. The tail was gently grasped by hand and rats were suspended 50 cm above their home cages. Complete crossing or bending of each hind limb was scored as 3 and bending of one of the hind limbs was scored as 2. The angle of the hind limbs was scored as 1 if smaller than 90° and as 0 if greater than 90°.

Statistics

Statistical analysis was conducted in Excel (Microsoft, Redmond, WA, USA) with the add-in software Statcel (OMS, Tokyo, Japan). Data are expressed as the mean ± SEM. Differences between multiple datasets were assessed using two-factor repeated-measure analysis of variance with post-hoc Tukey-Kramer test. All data were collected and analyzed using a double-blind approach.

RESULTS

MeHg-induced weight loss and hind-limb crossing

Weight loss is a common effect of exposure to toxicants, and hind-limb crossing is characteristic in rodents exposed to MeHg (Ralston et al., 2008; Heath et al., 2010; Fujimura et al., 2020). We measured these endpoints to confirm MeHg toxicity under our experimental conditions. MeHg-treated rats exhibited significant weight loss 9 days from the first exposure in comparison with controls, and this weight loss persisted throughout
the experimental period (Fig. 1A). Hind-limb crossing scores increased time-dependently but not in control rats (Fig. 1B). These findings suggest that our experimental conditions induced the characteristic phenotype in MeHg-exposed rats.

**MeHg exposure impairs pain sensation but no other sensory modalities**

We used stimulus-response behavioral analyses to assess sensitivity to MeHg by sensory modality. Pain sensation was analyzed using the foot shock test; significant hypoalgesia was observed in MeHg-exposed rats 11 days from the first MeHg treatment onwards, but responses returned to control levels after 48 days (Fig. 2A). Cold sensation was assessed using the tail immersion test. Slight hypesthesia was observed in MeHg-treated rats but the difference from responses in controls was not statistically significant (Fig. 2B). Heat and mechanical sensations were analyzed using the tail flick test and von Frey test, respectively; no hypesthesia or hyperesthesia were observed during the experimental period (Fig. 2C and D). Figure 3 displays the relative values of each sensory modality in MeHg-exposed rats. Even though a cold hypesthesia-like phenotype was observed in MeHg-exposed rats, statistical significance was only noted in the foot shock test (pain sensation). Taken together, these data suggest that pain sensation is the major sensory modality susceptible to MeHg poisoning under our experimental conditions.

**DISCUSSION**

In the present study, we investigated the sensitivity of various sensory modalities to MeHg exposure in rats using stimulus-response behavioral analyses. Our experimental conditions increased the threshold of the pain sensation (hypoalgesia); however, other sensory modalities were not affected. Furthermore, this MeHg-induced hypoalgesia recovered gradually after 7 weeks of daily experimentation. These results suggest that the pain sensation is the sensory modality most susceptible to MeHg in rats and that this hypoalgesia can be reversible.

We were unable to locate comparative studies of the sensitivity of sensory modalities to MeHg in either Minamata disease patients or MeHg-exposed laboratory animals. Interestingly, Fukuda et al. reported a high prevalence of subjective complaints of loss of the pain sensation in both men and women in a MeHg-polluted area who had not been diagnosed with Minamata disease. However, there were no subjective complaints of loss of thermal sensations, and complaints of loss of the touch sensation were recorded only in women (Fukuda et al., 1999). These and our findings suggest that susceptibility to the pain sensation following MeHg exposure may be relatively higher than to other sensory modalities in both humans and rats.

Under our experimental conditions, the loss of the pain sensation recovered to control levels 36 days after the end of MeHg treatment. In humans, MeHg-exposed cases have been shown to recover some neurological func-
tionality in both the short (Amin-zaki et al., 1978) and long term (Uchino et al., 2005), especially if the symptoms were mild (Snyder and Seelinger, 1976). In addition, MeHg-exposed rats also exhibit gradual restoration of rotarod performance, which requires the recovery of sensory feedback (Sakamoto et al., 1993). Even though these studies did not report the recovery of the pain sensation itself, the fact of the recovery of several neurological symptoms may support our findings.

The mechanism underlying the sensory disruption induced by MeHg is thought to be an impairment of the central and/or peripheral nervous systems. Minamata disease patients exhibit drastic neural degeneration and atrophy in the somatosensory cortex (Eto and Takeuchi, 1978; Ekino et al., 2007; Jackson, 2018), and severe axonal degeneration, myelin degradation, and degeneration of neurons in dorsal root ganglia are predominantly observed (Eto and Takeuchi, 1978; Takeuchi et al., 1978; Eto et al., 2002). Although the impairment of both central and peripheral nervous systems can cause the sensory disruption induced by MeHg, there are several lines of evidence that suggest the central nervous system (somatosensory cortex) as a causal region of MeHg-induced sensory disturbance. For example, in an electrophysiological study, the sensory velocity, sensory threshold, and H reflex were unaffected in MeHg-poisoned patients (Le Quesne et al., 1974; Von Burg and Rustam, 1974), and damage to peripheral sensory nerves may not be the cause of late sensory symptomatology (Snyder and Seelinger, 1976). In contrast, Minamata disease patients exhibit peripheral neuropathy (Eto and Takeuchi, 1978; Takeuchi et al., 1978; Eto et al., 2002). The lesser involvement of

Fig. 2. Hypoalgesia and recovery following exposure to methylmercury (MeHg). (A) Foot shock test. (B) Tail immersion test. \( n = 10 \) and 11 for controls and MeHg-exposed rats, respectively. (C) Tail flick test. (D) von Frey test. \( n = 10 \) each for controls and MeHg-exposed rats. Data were binned every 2 days. Separated thick bars marked “MeHg” indicate MeHg administration. Data are means ± SEM. Two-factor repeated-measure analysis of variance with post-hoc Tukey-Kramer test. ** \( p < 0.01 \) compared with controls.
the peripheral nervous system in some sensory impairment cases may be the result of a relatively short exposure (1-2 months) compared with the longer exposure in Minamata disease cases (several months to years) (Bakir et al., 1980). Even though a few cases of peripheral injury have been reported (Snyder, 1972; Rustam et al., 1975), the sensitivity to MeHg associated with the sensory system may be relatively higher in the central nervous system in humans.

In MeHg-exposed rodents, severe pathological changes in the central and peripheral nervous systems, which can be related to sensory disruption, have been reported. In the central nervous system, marked neuronal degeneration in the cerebral cortex was observed in MeHg-exposed rodents (Glaser et al., 2013; Feng et al., 2017; Yang et al., 2020). In the peripheral nervous system, extensive neuronal degeneration in dorsal root ganglia (Delio et al., 1992; Sakamoto et al., 1998; Cao et al., 2013; Shinoda et al., 2019b) and damage to sensory axons (Cavanagh and Chen, 1971; Miyakawa et al., 1974; Yip and Chang, 1981; Arimura et al., 1988; Cao et al., 2013; Shinoda et al., 2019b) and myelin (Miyakawa et al., 1970; Munro et al., 1980; Yip and Chang, 1981; Cao et al., 2013) have been reported. MeHg-exposed rats also exhibited decreased amplitudes in the peripheral nervous system, but the latencies were normal for the potentials evoked in the spinal dorsal roots (Arimura et al., 1988). Furthermore, spindle afferents and Golgi tendon organ sensory fibers degenerated completely in MeHg-exposed rats (Yip and Riley, 1987). In swine, MeHg exposure caused neuronal degeneration in both the central and peripheral nervous systems (Charlton, 1974). Although the problem of cross-species extrapolation and differences in experimental conditions are important considerations (Rees et al., 1990; Winneke, 1992; Rice, 1996), both animals and humans exhibit central and peripheral neural dysfunction following exposure to MeHg. Therefore, impairment of these systems is still considered the mechanism of MeHg-induced sensory disruption, especially in chronic exposures.

One of the possible mechanisms of the high susceptibility of pain sensation to MeHg is the different sensitivity of peripheral sensory neurons to MeHg. We previously reported that drastic neuronal degeneration was observed in dorsal root ganglion at 14 days after the first MeHg administration in the same experimental condition (Shinoda et al., 2019b). Besides, we also discovered selective degeneration of relatively small to medium size neurons (the diameters were 30.9-47.2 µm, data not shown), which is known to include the sensory neurons relaying painful signals (Esposito et al., 2019). We did not check the morphological and functional alteration of the spinal cords and somatosensory cortex in the MeHg exposed rats. Therefore, further investigation is required to clarify the mechanism of pain sensation susceptibility to MeHg.

We performed stimulus-response behavioral experiments to assess the sensitivity to MeHg neurotoxicity of several sensory modalities in rats. We found that the pain sensation was more susceptible than other sensory modalities, but the underlying mechanism is still unclear and requires study.

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**Conflict of interest**---- The authors declare that there is no conflict of interest.
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