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Irreversible damage to auditory system functions caused by perinatal hypothyroidism in rats

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ABSTRACT

We examined the effect of perinatal hypothyroidism on auditory function in rats using a prepulse inhibition paradigm. Pregnant rats were treated with the antithyroid drug methimazole (1-methyl-2-mercaptoimidazole) from gestational day 15 to postnatal day 21 via drinking water at concentrations (w/v) of 0 (control), 0.002 (low dose), or 0.02% (high dose). Rats from methimazole-treated mothers were tested at ages 1, 6, and 12 months using techniques to examine prepulse inhibition and startle response. The startle stimulus consisted of 40 ms of white noise at 115 dB, whereas the prepulse, which preceded the startle stimulus by 30 ms, consisted of 20 ms of white noise at 75, 85, or 95 dB. When the prepulse intensity was 75 or 85 dB, the high-dose group showed decreased prepulse inhibition percentages compared with the control and low-dose groups. The reduced percentages of prepulse inhibition did not return to control levels over the 12-month study period. In contrast, no differences in prepulse inhibition were observed among the three dose groups when prepulse intensity was 95 dB. Moreover, the high-dose group displayed excessive reaction to auditory startle stimuli compared with the other groups. Reductions in plasma free thyroxine and body weight gain were observed in the high-dose group. We conclude that perinatal hypothyroidism results in irreversible damage to auditory function in rats.

1. Introduction

Thyroid hormones are essential for normal brain development because they regulate neuronal proliferation, migration, and differentiation (Porterfield, 1994, 2000). In pregnant women, thyroid hormone deficiencies result in cretinism in the offspring (Boyages and Halpern, 1993). The neurological features of cretinism include speech disturbances, mental retardation, gait disorders, and deafness. Patients with cretinism commonly display auditory deficits (Boyages and Halpern, 1993), suggesting that the auditory system is vulnerable to thyroid hormone deficiency (Goldey et al., 1995).

Auditory function can be tested with prepulse inhibition (PPI) or reflex modification. PPI involves inhibiting the startle response to an auditory stimulus with a high intensity pulse (P) when P is preceded by a non-startling, low-intensity stimulus prepulse (PP). The general procedure fixes the intensity of P and systematically varies the intensity of PP. The minimum intensity of PP that induces PPI is the auditory threshold for the effect of PP on the startle response (Crofton, 1990). PPI offers many benefits. First, PPI is applicable to early developmental stages in rats because the auditory startle response develops by postnatal day (PND) 12 (Schneider and Golden, 1986, 1987). Second, PPI does not require any preliminary training to acquire the startle response. Third, PPI can be achieved in mice, rats, rabbits, pigeons, and human infants and adults (Hoffman and Ison, 1980). Because the neurobiological processes of PPI are similar in mammalian species (Crofton, 1990), it may be possible to extrapolate the results from animal experiments to humans.

The relationships between thyroid hormone deficiency and auditory deficits have been demonstrated in animal models using PPI of the auditory startle response (Goldey et al., 1995; Henck et al., 1996; Schneider and Golden, 1986, 1987). Pregnant rats treated with antithyroid drugs such as methimazole (MMI) or propylthiouracil (PTU) give birth to pups with perinatal hypothyroidism. The auditory threshold of PP required to induce PPI is elevated in rats exposed to PTU (Goldey et al., 1995). In addition, MMI-treated rats have a delay in the acquisition of the startle response in a dose-dependent manner from PND 15.2 to 17.8 compared with control rats on PND 12 (Schneider and Golden, 1986). Perinatal hypothyroidism decreases the amplitude of the startle response on PND 17–24, whereas it enlarges the amplitude on PND 43 or 75 (Goldey et al., 1995; Henck et al., 1996).

However, the long-term effect of perinatal hypothyroidism on auditory function remains unresolved. PTU-treated rats show elevated auditory brainstem response (ABR) thresholds that do not recover 8–9 weeks after birth (Axelstad et al., 2008). MMI treatment also causes an ABR waveform with a slower latency and altered shape compared with controls even on PND 90 (Albee et al., 1989). Further studies are necessary to clarify whether perinatal hypothyroidism irreversibly damages auditory function.

In this study, we examined the long-term effect of perinatal hypothyroidism on auditory function using the PPI paradigm. Pregnant rats were treated with MMI and the pups were tested over a 12-month

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period after birth. We predicted that the auditory threshold required to induce PPI would be elevated and the amplitude of the startle response would be higher over the 12-month period.

2. Materials and methods

2.1. Subjects

Twenty-four pregnant Wistar rats were purchased at gestational day (GD) 8 from Japan SLC Inc. (Hamamatsu, Japan). The animals were housed in individual cages, supplied with the certified rat chow MF (Oriental Yeast Ltd., Sapporo, Japan) *ad libitum*, and randomly assigned to either a control group (n = 8), a low-dose group (n = 8), or a high-dose group (n = 8). The antithyroid drug MMI (Sigma Aldrich Co., MO, USA) was dissolved in distilled water and administered to the animals via drinking water. MMI at concentrations (w/v) of 0 (control), 0.002 (low dose), or 0.02% (high dose) was administered to the rats starting on GD 15 until PND 21. MMI treatments of 0.001–0.005% are the lowest doses that cause hypothyroidism in adult rats; hence, 0.002% MMI treatment was considered as the low dose in this study (Cooper et al., 1984). Moreover, treatment with 0.02% MMI from GD 15 to PND 21 affects myelinogenesis in rat brain (Barradas et al., 2000). Two pups (one male and one female) were sampled from each litter after weaning on PND 21. Pups in the high-dose group were weaned on PND 28 because of developmental delay. The mothers of these pups were given tap water from PND 22 to 28. Eight pups were assigned to each of the following groups: male control (MC), male low-dose (ML), male high-dose (MH), female control (FC), female low-dose (FL), or female high-dose (FH) groups. Two or three pups from each group were housed per cage. The certified rat chow MF and tap water were provided to the pups *ad libitum*.

Room temperature was maintained at 22 ± 2 °C with a relative humidity of 50 ± 10%. The rat pups were kept under a 12-h light/dark cycle (light, 19:00–07:00 h; dark, 07:00–19:00 h), and PPI and startle responses were analyzed during the dark period. This protocol was approved by the Center for Advanced Science and Technology (Hokkaido University). All conditions complied with the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

2.2. Apparatus

The experimental chamber was a clear acrylic cage (15 × 22 × 12 cm) with aluminum mesh walls on two sides. A piezoelectric accelerometer (GH313A, GA-245SO; KEYENCE, Osaka, Japan) attached underneath the experimental chamber detected movements of the rats and transduced them into voltage outputs. The voltage outputs were digitized at 1000 Hz and recorded on a personal computer through a 60 Hz low-pass filter. White noise was used as P and PP. The white noise was amplified by a speaker placed adjacent to the experimental chamber. Both the experimental chamber and the speaker were placed within a sound-insulated box to attenuate external sound and light. Background noise was maintained at a constant level of 70 dB throughout testing.

2.3. Auditory startle response and PPI

Rats were habituated to the experimental chamber in the presence of 70 dB continuous background noise for 5 min and then underwent both startle response and PPI testing. The rats were exposed to 115 dB of P for 40 ms for startle response testing. A startle response was defined as the difference between the maximum and minimum peak amplitudes of the voltage outputs within a 200-ms period after the onset of P. Startle response testing consisted of 10 trials per day for 3 days. The inter-trial intervals (ITIs) were varied, and the mean ITI was 20 s.

Rats were exposed to either P alone (P trial) or P with a preceding PP (PP trial) for PPI testing. A 115-dB 40-ms P was presented in P trials. PP with an intensity of 75, 85, or 95 dB for 20 ms was presented 30 ms before P in PP trials. The intensity of PP was changed daily for 3 days. Half of the rats received PPs with ascending intensities of 75, 85, and 95 dB, whereas the other half received PPs with descending intensities of 95, 85, and 75 dB. PPI testing consisted of a pseudo-random presentation of eight P trials and 10 PP trials per day for 3 days. The percentage PPI was calculated by the following formula: % PPI = [(P − PP)/P] × 100, where P and PP are averages of the startle response amplitudes of P and PP trials, respectively. The ITIs were varied, and the mean ITI was 20 s.

The rats were tested for PPI and startle responses for 3 days at 1, 6, and 12 months after birth. In other words, the rats were repeatedly tested at these three time points.

2.4. Determination of thyroid hormones

Free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were determined from the same set of MMI-treated animals. Whole blood was collected from the abdominal aorta of ether-anesthetized pups on PND 21, centrifuged at 3000 rpm for 10 min, and the plasma was stored in a microtube. The FT3 and FT4 concentrations were determined using the ACS-FT3 II and LKFT41 kits, respectively (Siemens Healthcare Diagnostics Co., Tokyo, Japan). TSH concentrations were determined using the rat TSH ELISA kit (R-type) (Shibayagi Co., Shibukawa, Japan). All assays were performed at Mitsubishi Chemical Medience (Tokyo, Japan).

2.5. Statistical analysis

The percentages of PPI were analyzed by a four-factor analysis of variance (ANOVA) between subject variables of dose and sex and within subject variables of PP intensity and age. Startle response amplitudes and body weights were analyzed by a three-factor ANOVA between subject variables of dose and sex and within subject variables of age. A two-factor ANOVA was used between subject variables of dose and sex to analyze plasma FT3, FT4, and TSH concentrations as well as body weights on PND 21. Ryan's method was used for multiple comparison tests when a primary effect was significant. These statistical analyses were executed using ANOVA 4 on the Web (http://www.hju.ac.jp/~kiriki/anova4/about.html).

3. Results

3.1. Percentage of PPI

The effects of dose, PP intensity, and age were significant [$F(2,42) = 16.385$, $p<0.001$; $F(2,84) = 52.342$, $p<0.001$; $F(2,84) = 24.608$, $p<0.001$]. An interaction was observed between dose and PP intensity [$F(4,84) = 9.991$, $p<0.001$]. When the PP intensity was 75 dB or 85 dB, the high-dose group showed decreased percentage PPI compared with both the control and low-dose groups ($ps<0.05$) (Fig. 1a). However, no significant difference was observed among the three dose groups when a PP of 95 dB intensity was presented. The interaction between dose and age was also significant [$F(4, 84) = 8.834$, $p<0.001$]. No significant difference in percentage PPI was observed among the three dosage groups at 1 month, but the high-dose group displayed lower percentage PPI than both the control and low-dose groups at 6 and 12 months ($ps<0.05$) (Fig. 1b). The control and low-dose groups showed an increased percentage PPI at 6 and 12 months compared with that at 1 month ($ps<0.05$), whereas the high-dose group did not show an increased percentage PPI over the 12-month period. No significant difference in percentage PPI was observed between the control and low-dose groups. Sex