EFFECTS OF PERINATAL THYROID HORMONE DEFICIENCY ON ULTRASONIC VOCALIZATION IN RAT PUPS

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Abstract

Perinatal thyroid hormone deficiency causes irreversible damage to auditory system functions. Hearing deficits have potential to affect the ultrasonic vocalizations (USVs) of rat pups on separation from the mother rat. In this study, we examined the effects of perinatal thyroid hormone deficiency on the emission of 40 kHz USVs by rat pups. Pregnant rats were treated with the antithyroid drug methimazole (MMI) from gestational day 15 to postnatal day (PND) 21 administered through drinking water. The concentrations of MMI (w/v) were 0% (control group), 0.01% (low-dose group), and 0.015% (high-dose group). The USVs were recorded during 5 min of maternal separation on PND 3, 6, 9, 12, 15, 18, and 21. During PND 3–12, all the three dose groups exhibited USVs at frequencies of 40–50 kHz. Thus, MMI did not affect USVs of pups. After PND 15, the USVs declined and almost disappeared by PND 21. However, on PND 15, the low-dose and high-dose groups produced more USVs at frequencies of 40–45 kHz than the control group. The high-dose group produced even more USVs on PND 18 than the control and low-dose groups. Therefore, we conclude that perinatal thyroid hormone deficiency has potential to affect the emission of USVs because of hearing deficits.

Introduction

Perinatal thyroid hormone deficiency causes irreversible damage to auditory system functions.[9] Many of the outer hair cells are lost, and the afferent and efferent innervations to the hair cells degenerate.[8] Severe hearing deficits have been detected in a wide range of tone frequencies from 1 to 40 kHz.[1] Auditory system dysfunctions can affect social behavior in rats because rats emit ultrasonic vocalizations (USVs) for interactions with others. For example, rat pups emit 40 kHz USV when they are separated from the mother rats.[3) 4) 5)] In response, the mother rats approach the pups and exhibit retrieval behavior.[5] This study aims to examine whether perinatal thyroid hormone deficiency affects the USV emissions of rat pups.

Materials and methods

Thirty pregnant Wistar rats were purchased on gestational day (GD) 14. The pregnant rats were housed in individual cages and randomly assigned to a control group (n = 10), low-dose group (n = 10), or high-dose group (n = 10). The antithyroid drug methimazole (MMI) was dissolved in distilled water and administered through drinking water to the pregnant rats from GD 15 to postnatal day (PND) 21. The concentrations of MMI (w/v) were 0% (control), 0.01% (low dose), or 0.015% (high dose). The pups were culled to four males and four females per litter on PND 2. Four male and four female pups from different mothers in each dose group were used to obtain USV recordings. The USV recordings were obtained by individually separating the pups from their mothers for 5 min before returning them to their mothers for an equal period of time (5 min). The pups were again separated and their USV emissions were recorded for 5 min.[6] After USV recording, they were returned to their mothers. The recordings were obtained on PND 3, 6, 9, 12, 15, 18, and 21 using a Sonotrack system (METRIS, Hoofddorp, the Netherlands).

Room temperature was maintained at 22 ± 2°C with a relative humidity of 50 ± 10%. The mother rats and pups were maintained with a 12 h light/dark cycle (light, 19:00–07:00 h; dark, 07:00–19:00 h). The USV emissions were recorded during the dark period. The mother rats were provided rat chow and tap water *ad libitum*. This protocol was approved by Hokkaido University, and all conditions complied with the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

The number and duration of USV emissions were analyzed using three-factor analysis of variance (ANOVA) for the between-subject dose variable, within-subject variables of PND, and the USV frequency. The USV frequencies were classified into eight classes, i.e., <35, 35–40, 40–45, 45–50, 50–55, 55–60 60–65, and >65 kHz. The body weights were analyzed using two-factor ANOVA for the between-subject dose variable and

within-subject PND variable. Ryan's method was used for multiple comparison tests if the primary effect was significant. These statistical analyses were executed using web-based ANOVA4 (http://www.hju.ac.jp/~kiriki/anova4/about.html). Four control pups (three male and one female) were dead during PND 15–18; therefore, other control pups were supplemented on PND 21. Statistical analyses were performed on PND 3–12. The data collected during PND 15–21 were analyzed separately.

Results

The number of USV emissions differed significantly among PNDs [$F(3, 54) = 11.800$, $p < 0.001$]. The USV emissions on PND 6 and 9 were higher than those on PND 3 and 12 ($p < 0.05$). The USV emissions during PND 3–12 were pooled and classified according to the eight frequency classes (Fig. 1). The number of USV emissions in each frequency class differed significantly [$F(7, 126) = 67.490$, $p < 0.001$]. Those at frequencies of 40–50 kHz were increased compared with those at other frequencies ($p < 0.05$). The MMI dose did not affect the number of USV emissions.

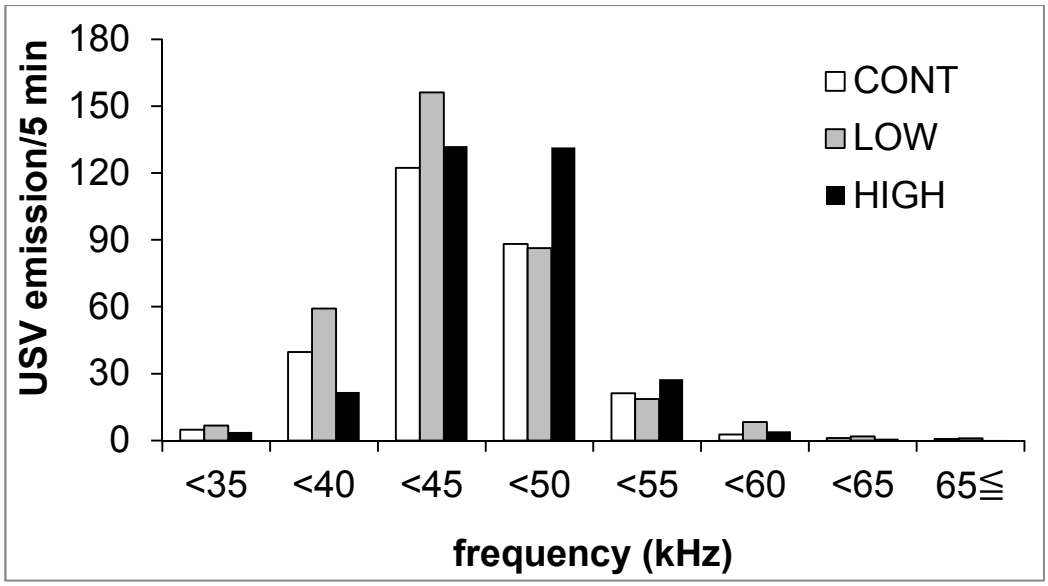

Fig. 1. The number of USV emissions in the eight frequency classes during PND 3–12. The number of USV emissions at frequencies of 40–50 kHz was greater than that at other frequencies ($p < 0.05$). The MMI dose did not affect the number of USV emissions.

The duration of USV emissions differed significantly among PNDs [$F(3, 54) = 10.807$, $p < 0.001$]. The durations during PND 6–12 were longer than those on PND 3 ($p < 0.05$). The USV emissions during PND 3–12 were pooled and classified according to the eight frequency classes (Fig. 2). The duration of USV emissions in each frequency class differed significantly [$F(7, 126) = 162.075$, $p < 0.001$]. The durations were longer at frequencies of 40–50 kHz than at other frequencies ($p < 0.05$). The MMI dose did not affect the duration of USV emissions.

On PND 15, the MMI dose had a significant effect on the number and duration of USV emissions [$F(2, 16) = 5.335$, $p < 0.017$; $F(2, 16) = 7.148$, $p < 0.007$]. The number and duration of USV emissions were higher in the low-dose and high-dose groups than in the control group ($p < 0.05$). The relationship between the MMI dose and frequency was significant with respect to the number and duration of USV emissions [$F(14, 112) = 4.338$, $p < 0.001$; $F(14, 112) = 2.47$, $p < 0.005$]. The low-dose and high-dose groups produced more USV emissions at frequencies of 40–45 kHz compared with the control group (Fig. 3) ($p < 0.05$). The duration of USV emissions of the low-dose and high-dose groups was prolonged at frequencies of 35–50 kHz compared with that of the control group ($p < 0.05$). The MMI dose significantly affected the number of USV emissions on PND 18 ($F(2, 14) = 10.721$, $p < 0.002$). The high-dose group produced more USV emissions than the control and low-dose

groups ($p < 0.05$). There was a significant relationship between the MMI dose and frequency [$F(14, 98) = 2.751$, $p < 0.002$]. The high-dose group produced more USV emissions at frequencies of 35–50 kHz compared with the control and low-dose groups ($p < 0.05$). After PND 15, the number of USV emissions declined in all the groups and almost disappeared by PND 21.

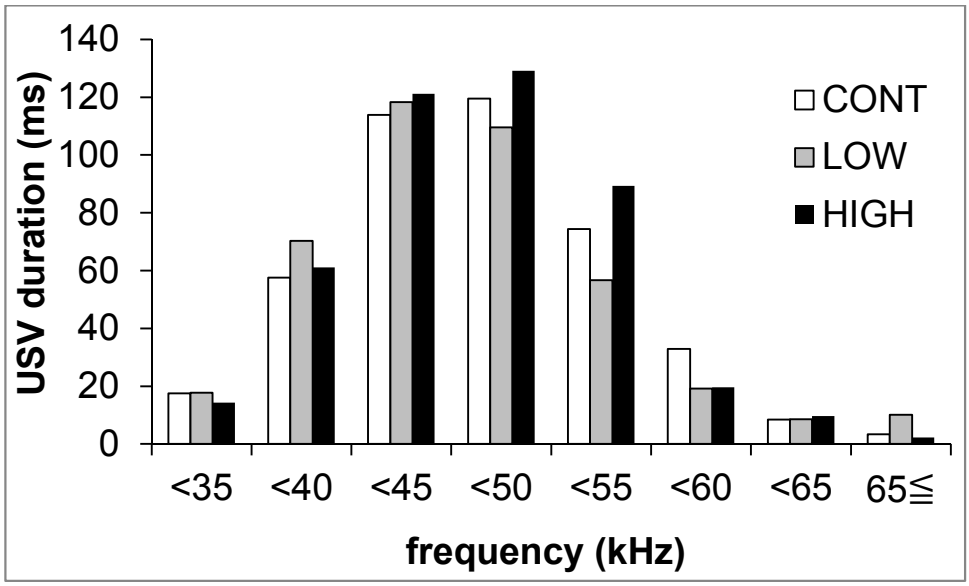

Fig. 2. The duration of USV emissions in the eight frequency classes during PND 3–12. The duration of USV emissions at frequencies of 40–50 kHz was longer than that at other frequencies ($p < 0.05$). The MMI dose did not affect the duration of USV emissions.

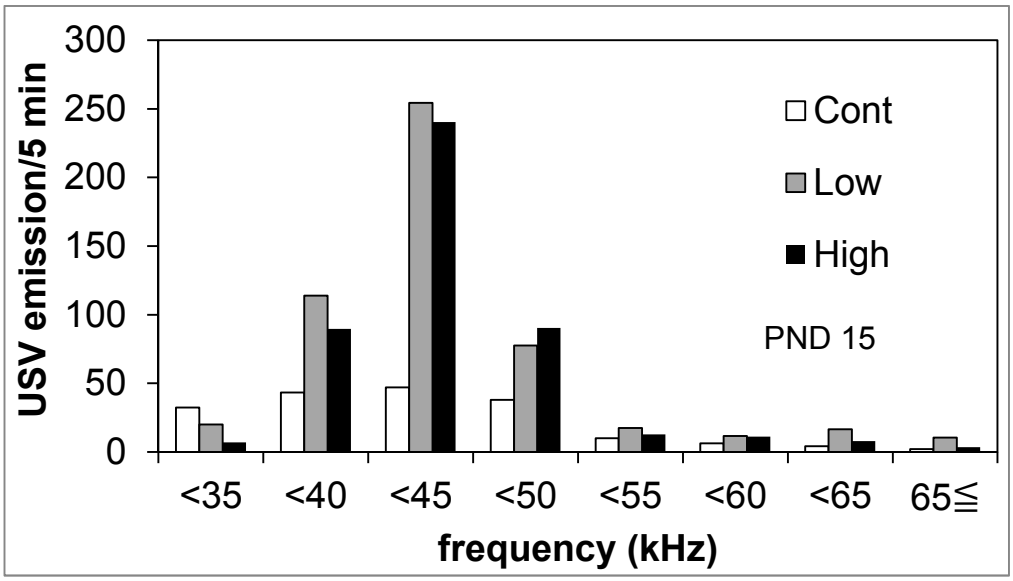

Fig. 3. The number of USV emissions in eight frequency classes on PND 15. The low-dose and high-dose groups had increased USV emissions at frequencies of 40–45 kHz compared with the control group ($p < 0.05$).

Body weight gains were not affected by the MMI dose during PND 3–12. However, the body weights on PND 21 were significantly different among the MMI dose groups [$F(2, 18) = 4.404$, $p < 0.028$]. The high-dose group had lower body weight gains compared with the control and low-dose groups ($p < 0.05$).

Discussion

The present study suggests increased USV emissions by pups on maternal separation. The number of USV emissions increased during PND 3–9, declined after PND 12, and almost disappeared by PND 21 when the rat pups were capable of weaning. The USV emissions were higher at frequencies of 40–50 kHz, and their durations were 100–140 ms. These characteristics of the USV emissions were in accordance with those reported by previous studies.[3) 4) 5) 7)] The number and duration of USV emissions did not differ among the three dose groups during PND 3–12. Thus, perinatal thyroid hormone deficiency did not affect the production of USV emissions during maternal separation.

However, the effects of thyroid hormone deficiency were evident after PND 15. Both the low-dose and high-dose groups produced more USV emissions at frequencies of 35–50 kHz. Perinatal thyroid hormone deficiency causes irreversible damage to auditory system functions. Hypothyroid rats have an elevated auditory threshold at high frequency tones from 1 to 40 kHz.[1)] The USVs produced by rat pups occur at frequencies around 40 kHz. Thus, the thyroid hormone-deficient groups in this study may have not been able to hear their own USV emissions, thereby increasing the number of USV emissions.

The mother rats approached their pups on USV emission by the pups and exhibited retrieval behavior.[5)] The mother rats exposed to perinatal thyroid hormone deficiency may not have exhibited maternal behavior, such as approach and retrieval behavior, because of hearing deficits, even when the pups emitted USVs. Thus, USV emissions are considered to be the communication tools for adult rats. For example, adult rats emit 22 kHz USVs when they experience aversive or threatening stimuli such as predators, whereas 50 kHz USVs are emitted when they approach rewards or mating partners.[11)] Thus, hearing deficits may affect the social behavior maintained through USV emissions.

On the other hand, perinatal thyroid hormone deficiency delays the development and affects physiological landmarks such as body weight gain, hair cover production, incisor eruption, and eye opening.[2) 10)] In this study, the high-dose group had a lower body weight gain on PND 21. Some of the high-dose group members exhibited delayed eye opening, whereas the control and low-dose groups exhibited eye opening by PND 18. Thus, the high-dose group may have required maternal care for a longer period because of their delayed development, thereby increasing their USV emissions.

We were not able to analyze the USV data with the same animals during PND 3–21 because some of the control pups died. The USV data on PND 15 and 18 lacked two or three control pups. Additional control pups were introduced but they underwent maternal separation only on PND 21. Further studies are required to confirm our results.

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