THE DEFICIT OF BEHAVIOURAL INHIBITION IS NOT IMPROVED BY METHYLPHENIDATE IN HYPOTHYROID RATS

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Abstract

It is a common knowledge that dioxins and Poly-Chlorinated Biphenyls (PCBs) disrupt thyroid hormone systems that are essential for normal developments of the central nervous system (CNS). Exposures to these chemicals will cause behavioral alterations similar to the symptoms of Attention-Deficit/Hyperactivity Disorder (AD/HD). By means of a target detection task, the present study focused on the disruption of thyroid hormone systems by organic chlorinated compounds and examined whether perinatal hypothyroidism causes attention deficits, which is one of the main symptoms of AD/HD. No significant hypothyroid effects were observed regarding the percentages of correct responses. However, hypothyroid rats showed more perseveration in their responses. Moreover, methylphenidate could not improve these responses of the hypothyroid rats. This indicates that hypothyroidism during CNS developments does not cause attention deficits but causes a deficit of behavioural inhibition based on the mechanisms that are different from those of AD/HD.

Introduction

Dioxins and Poly-Chlorinated Biphenyls (PCBs) exert their behavioural toxicities through the disruption of thyroid hormone systems. Since the structures of these organic chlorinated compounds and thyroid hormones are similar, dioxins and PCBs can bind the thyroid hormone receptors and carrier proteins, and disturb thyroid hormone systems[6,7]. Thyroid hormone is essential for proper developments of the central nervous system (CNS); disruption of the hormone systems during CNS developments can cause neurological dysfunctions and lead to behavioural alterations. The behavioural characteristics induced by dioxins and PCBs are similar to the symptoms of Attention-Deficit/Hyperactivity Disorder (AD/HD). AD/HD is one of the most common childhood disabilities characterised by hyperactivity, impulsiveness, and attention deficits[1]. Although the causes of AD/HD are not clear, organic chlorinated compounds are suspected to be the possible risk factors due to its behavioural toxicities mediated by the disturbance of thyroid hormone systems. Laboratory studies have shown that hypothyroidism during CNS developments causes the increases of locomotion and exploratory behaviour in rats[3-5]. Furthermore, the rat treated with PCBs display impulsive bahaviour in a Fixed Interval (FI) schedule as well as a Differential ReinForcement of low rate (DRL) schedule[2].

It is therefore likely that hypothyroidism induced by organic chlorinated compounds is one of the risk factors for AD/HD. However, there is little information whether hypothyroidism causes attention deficits. In the present study, we examined effects of hypothyroidism on attentional abilities in rats. Additionally, we administered methylphenidate (MPH), one of the

most common drugs for AD/HD, to hypothyroid rats to verify the predictive validity.

Materials and Methods

Twenty four pregnant rats of Wistar strain were purchased on gestational day 8. These animals were individually housed and randomly assigned to three groups, (ⅰ) C (Control), (ⅱ) L (Low concentration), and (ⅲ) H (High concentration). Rats in L and H groups were treated with methimazole (MMI) at concentrations (w/v) of 0.01% and 0.02%, respectively. MMI was dissolved in distilled water and given to dams through drinking water from gestational day 15 to postnatal day (PND) 21. At the time of weaning (PND21), one male and one female offspring were sampled from each dam and placed in a cage that house 2 to 3 rats. Male and female rats were housed separately. Each concentration group consisted of eight male and eight female offspring. These rats from the three groups were labeled as CM (Control-Male), LM (Low-Male), HM (High-Male), CF (Control-Female), LF (Low-Female), and HF (High- Female). The rats had free access to food until 12 weeks of age. Then all the rats were placed under food restrictions and maintained according to their free-feeding body weights; 85% for the male rats and 90% for the female rats, respectively. The room temperature was maintained at 22±2°C and the relative humidity was 50±10% under a 12-h light/dark cycle (dark, 07:00-19:00 h). The behavioral tests were conducted in a dark period. This research was carried out with the approval of The Center for Advanced Science and Technology (Hokkaido University). The environmental conditions complied with The Guide for the Care and Use of Laboratory Animals (Hokkaido University).

Five standard operant chambers were used for the present experiment. After the shaping of lever pressing, all animals were trained under a Discrete Trial-Continuous ReinForcement (DT-CRF) schedule. A trial started when a signal light (target) was switched on. If rats pressed the lever during the target presentation, a food pellet was delivered as a reward. Then, the target was switched off and an inter-trial interval (ITI) began. During ITI, responses did not yield any reward. The ITI was gradually increased from 0s to 10s. DT-CRF training consisted of 50 trials per day and continued for 7 consecutive days. After DT-CRF training, a target detection task was conducted. The target was presented after variable intervals (VIs) in four time periods, 10, 17.4, 30, and 52.5 s, respectively. These intervals were presented pseudo-randomly with equal probability. If the rat pressed the lever within the limited hold (LH) period (4s), a food pellet was delivered and the target was switched off. This response was considered to be a hit response. If the rats did not press the lever within the LH period, no food pellet was delivered and the target was switched off. The responses within 10 s after the target removed were considered to be perseverative responses, or responses that exhibit perseveration. Following the VIs, the next trial began. During VIs, responses did not yield any reward. Training for target detection consisted of 80 trials per day and continued for 11 days. Animals were injected with saline and methylphenidate 20 minutes prior to the task. Saline was administered to rats from the Day 1 to Day 5 and from Day 9 to Day 11 of the task. MPH (4mg/kg) was administered from Day 6 to Day 8. The experiment and data recording were controlled by a personal computer.

Percentages of hit responses and number of perseverative responses were analyzed by a three-way ANOVA (MMI concentration × MPH dose × VI). Since body weights of the male were significantly heavier than those of female groups, their data were analyzed separately. We analyzed the data of the first 40 trials, for the gradual decreases of hunger drive were expected in daily training due to deliveries of food pellets as rewards.

Results and Discussion

For the male groups, a three-way ANOVA did not reveal the main effects of MMI concentrations and MPH dose on the percentages of hit responses, along with the interaction of MMI concentrations × MPH dose. A three-way ANOVA of persevarative responses revealed significant main effects of MMI concentrations ($F(2,19) = 3.68$), and HM rats exhibited more perseverative responses compared with other groups ($p<0.05$; Fig. 1). There were no significant effects of MPH dose and interaction of MMI concentrations × MPH dose on the perseverative responses. As in the male group, a three-way ANOVA did not reveal the main effects of MMI concentrations and medication on the hit percentages, along with the interaction of MMI concentrations × MPH dose for female groups. There was no significant effect of MMI concentrations on perseverative response in the female group.

In the present experiment, the hit percentage was not significantly different between each concentration groups in both the males and the females. Rats had to pay attention to target presentation in order to press the lever within the LH period; hit percentages reflected the attentional abilities of rats. Since no significant decreases of hit percentages were obtained in the hypothyroid group, the hypothyroid states during CNS developments did not cause attention deficits. However, the possibilities remain that no significant differences of hit percentages between the concentration groups were derived from a ceiling effect. If the LH period (4 s) were either too long or not challenging for rats, the higher hit percentages of each group would not reflect their attentional abilities appropriately. To elucidate this problem, further studies in a shorter LH period (e.g. 2 s or 1 s) is required. On the other hand, HM rats produced more persevarative responses than the other groups. The responses during VIs were not related to the reward. In particular, the responses immediately after the target removed should be inhibited because the reward was already delivered. HM rats were not able to inhibit these inappropriate responses and continue to perseverate on lever pressings, suggesting that HM rats had a deficit of behavioural inhibition, the ability to inhibit and control responses. Barkley advocates the theory that AD/HD comprises a deficit in behavioural inhibition[1]. According to his theory, HM rats have characteristics as a model of AD/HD. However, MPH did not improve persevarative responses of HM rats, even though MPH is one of common drugs for AD/HD. This implicates that the deficit of behavioural inhibition in HM rats may be based on the neural mechanisms different from those of AD/HD. Moreover, the lack of MPH effects reveals that there was little predictive validity of hypothyroid rats as an AD/HD model.

In conclusion, hypothyroid states during CNS developments would not cause attention deficits but cause deficit of behavioural inhibition. The deficit is based on the different neural mechanisms from those of AD/HD due to the lack of the

MPH effects. These results indicate that the hypothyroid rats have characteristics of AD/HD partially, but not perfectly.

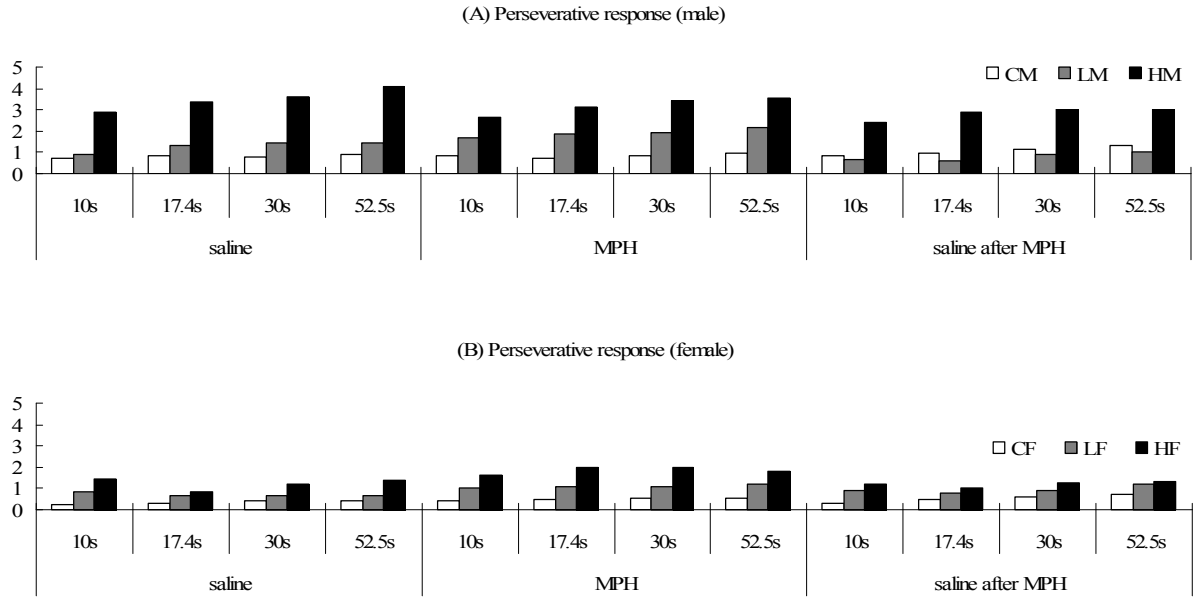

Fig.1. The number of perseverative responses in Limited Hold period (4 s). Data of male rats is shown on the section (A) and fdata of female rats is shown on section (B). HM rats exhibit more perseverative responses than the other groups (p<0.05). There is no significant difference of the number of responses for female groups.

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