Introduction

The volatile organic compound 1-bromopropane (CH₂-CH₂-CH₂Br; 1-BP), a substitute for specific chlorofluorocarbons, is mainly used in degreasing solvents and spray adhesives. It has been reported that occupational exposure to 1-BP causes neurotoxicity, such as numbness, gait disturbance, prolongation of distal latency and memory dysfunction [1]. Animal models exposed to 1-BP have also shown central neurotoxicity, including ataxic gait, prolongation of distal latency, alteration of mRNA levels of neurotransmitter receptors [1], and hippocampal disinhibition [2]. In vitro studies have revealed that the direct application of 1-BP enhanced the currents mediated by the activation of A type γ-aminobutyric acid (GABAₐ) receptors, suppressed the currents mediated by neuronal nicotinic acetylcholine receptors, and potentiated feedback inhibition in the cornu ammonis 1 (CA1) subfield of hippocampal slices [3]. The gene expression of the B-cell lymphoma-extra large molecule (Bcl-xl), and the activity of nuclear factor-kappa B (NF-κB), were suppressed in in vitro and in vivo studies [4]. The developmental effects of 1-BP have also been investigated [5], but little is known about the developmental neurotoxicity in offspring.

Prenatal Exposure to 1-Bromopropane Suppresses Kainate-Induced Wet Dog Shakes in Immature Rats

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Abstract: 1-Bromopropane (1-BP) is used in degreasing solvents and spray adhesives. The adverse effects of 1-BP have been reported in human cases and adult animal models, and its developmental toxicity has also been reported, but its effects on developmental neurotoxicity have not been investigated in detail. We evaluated the effects in rat pups of prenatal exposure to 1-BP on behaviors such as scratching and wet dog shakes (WDS), which were induced by injection of kainate (KA). Pregnant Wistar rats were exposed to vaporized 1-BP with 700 ppm from gestation day 1 to day 20 (6 h/day). KA at doses of 0.1, 0.5, and 2.0 mg/kg were intraperitoneally injected into a control group and a 1-BP-exposed group of pups on postnatal day 14. There was no significant difference in scratching between the control and the prenatally 1-BP-exposed groups, while suppression of the occurrence ratio of WDS was observed at the low dose of 0.1 mg/kg of KA in the prenatally 1-BP-exposed pups. Our results suggest that prenatal exposure to 1-BP affects neurobehavioral responses in the juvenile period.

Keywords: 1-bromopropene, prenatal exposure, developmental neurotoxicity, wet dog shake, rats.

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In our previous study of the developmental neurotoxicity of 1-BP, prenatal exposure to 1-BP altered hippocampal excitability and the gene expression of the Na⁺ channel [6] and glutamate receptor subunits on postnatal day (PND) 14 [7]. These results raised the possibility that prenatal exposure to 1-BP affects brain development and its related behaviors. However, conventional behavioral tests for rodents are difficult to apply to pups. Thus, we focused on the particular behaviors of scratching and wet dog shakes (WDS), which can be observed in pups.

Scratching is defined as repetitive and quick flexion-extension movements of the hind limbs toward the neck or the head region. This behavior has been shown to be spontaneously induced in normal as well as pathological conditions and is used as an itch model in rodents [8], although the behavior in pups remains to be analyzed. WDS is characterized as brief and fierce shaking of the head, neck, and trunk, appearing when rodents are wet, as the name suggests [9]. Interestingly, it has been reported that both scratching and WDS can be induced by electrical stimulation of limbic structures [10], and by several pharmacological interventions, such as kainate (KA) [9, 11] and pentylentetrazole. KA is the agonist of ionotropic glutamate receptors, which mediate excitatory neurotransmission and are predominantly distributed in the hippocampus, inner lamina of the neocortex, and ventral thalamus [12]. Thus, scratching and WDS induced by KA could be useful indices of changes in the excitatory neurotransmission of neuronal networks in pup brains. In this study, we examined the effect of prenatal exposure to 1-BP on behaviors in pups by evaluating the incidences of scratching and WDS induced by KA.

Materials and Methods

Animals and 1-BP inhalation

Thirty-two female and 16 male Wistar rats (designated the parental (P) generation) purchased from Kyudo Co. (Tosu, Japan) at 11 weeks of age were housed in plastic cages with paper-made chips (ALPHA-dri, Shepherd Specialty Papers, Milford, USA) on a 12 h light/dark cycle (light period: 07:00-19:00). The room temperature was kept at 23 ± 1°C. The relative humidity was about 70%. The animals had free access to food and water. Proestrus stage was verified with an impedance checker (MK-10B, Muromachi Kikai Co., Ltd., Tokyo, Japan). When the impedance was over three kΩ, the F0 female rats were mated with male rats. In the morning of the following day, the existence of sperm in the vaginal smear or vaginal plug was verified as the gestation day (GD) 0. Fourteen dams from the colony were used in the experiment. The pregnant rats of the P generation were randomly divided into two groups (7 rats in each): one group as the control and the other for exposure to 1-BP.

1-BP was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). Seven dams were exposed to 1-BP vapor at a concentration of 700 ppm (6 h/day) for 20 days from GDs 1 to 20 in an exposure chamber [13], whereas the other seven dams were provided fresh air in the same type of chamber. Both P generation groups were not allowed access to food and water during the inhalation period. Four weeks of 1-BP inhalation (700 ppm) resulted in apparent effects on the hippocampus in the adult rats [2]. Therefore, we first chose the concentration of 700 ppm to study the possible underlying mechanism of developmental neurotoxicity in prenatally 1-BP-exposed rats. The concentration of 1-BP was monitored with a gas chromatograph (GC353B FSL, GL Sciences Inc., Japan) equipped with a flame ionization detector.

All the pregnant rats gave birth to offspring (termed the first filial (F1) generation) on GD 21. The day of birth was defined as PND 0. We randomly gathered 26 F1 rats from the 7 control litters and 22 F1 rats from the 7 1-BP-exposed litters. All the F1 pups were bred with their mother rats during the lactation period. In this study, 24 female and 3 male F1 rats were obtained from the 7 dams in the control group, and 18 female and 4 male F1 rats were obtained from the 7 dams in the 1-BP-exposed group, respectively. We examined the F1 rats for the general toxicity of 1-BP inhalation exposure, such as litter size, sex ratio, testicular descent, vaginal opening, ear opening, and survival rate. The body weight of the F1 rats was measured on PND 14.

KA administration and behavioral observation

KA was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). KA (0.1, 0.5, and 2.0 mg/kg) was dissolved in phosphate buffered saline (PBS).
PBS or KA was intraperitoneally injected to the F1 rats at PND 14, after which the F1 rats were placed in a clear plastic cage, and the scratching and WDS were observed by video-recording for 180 min in a room for the behavioral observation. The room temperature was kept at about 25°C. The behavioral observation was conducted for 180 min between 09:30 and 15:30. The number of F1 rats that showed the scratching and the WDS behavior was counted and then the occurrence ratio was calculated. The duration and frequency of scratching and WDS were also measured. This experiment was approved by the Ethics Committee for Animal Care and Experimentation in accordance with the University of Occupational and Environmental Health, Japan.

All the chemicals used in this study were a reagent grade and purchased from commercial sources.

Statistical analysis

The difference in bodyweight between the F1 control and F1 1-BP-exposed groups was analyzed by Student $t$-test. The Mantel-Haenizel procedure was utilized to see the whole effect of the prenatal inhalation of 1-BP on the occurrence ratio of scratching and WDS. When appropriate, Fisher’s exact test determined significant differences. A two-way analysis of variance (ANOVA) was performed to clarify the effects of prenatal exposure to 1-BP and/or a dose of KA on the frequency and the duration of scratching and WDS. When appropriate, post hoc analysis by Scheffe’s test determined significant differences, respectively. The criteria of significant difference was $P < 0.05$ in all the statistical analyses. Data represent mean $\pm$ standard error of the mean (SEM).

Results and Discussion

General toxicity of 1-BP inhalation exposure in F0 and F1 generations

There were no outward pathological signs related to 1-BP in the F0 rats. The body weights of the P generation dams treated with 1-BP were not significantly different from those in the control (fresh air) group (data not shown). None of the F1 rats died during the experimental period, indicating that the exposure seemed to cause little stress on the dams in this study. There was no difference in the sex ratio, survival rate, or other clinical signs between the F1 control and F1 1-BP-exposed groups, with the exception of body weight. The body weight in the female F1 1-BP-exposed group ($32.5 \pm 0.5$ g) was significantly lower ($P < 0.01$, Student $t$-test) than that in the female F1 control group ($35.0 \pm 0.4$ g). The 1-BP-exposed male F1 rats also had a lower body weight ($33.5 \pm 0.3$ g) compared to the male F1 control group ($37.0 \pm 0.8$ g) ($P < 0.01$, Student $t$-test). Our results were consistent with previous studies showing that prenatal exposure to 1-BP has no effects on postnatal survival rate, excluding the body weight [5].

Effect of prenatal exposure to 1-BP on behavioral responses

KA administration elicits immobilization, followed by scratching, WDS, forelimb clonus, and status epilepticus (continuous chronic-tonic posturing of all 4 limbs) [11]. It is also known that a low dose less than 3 mg/kg of KA elicits scratching and WDS but hardly ever elicits epileptic convulsions. Our preliminary study also showed that doses of KA higher than 4 mg/kg induced convulsive behaviors as well as scratching and WDS, thus we chose doses of 0.1, 0.5, and 2.0 mg/kg of KA.

Behavioral data obtained from both genders is combined in Tables 1 and 2, because it has been reported that there are no sex differences in KA induced-behaviors in pups [14]. In the F1 control group, all of the tested pups showed scratching during the 180 min after injection of PBS or KA. The frequency and duration of the scratching was significantly higher only at the dose of 2.0 mg/kg (Table 1). WDS were observed in 80% of the PBS-injected control pups and in all of the KA-injected control pups. A significantly higher frequency of WDS was observed at the dose of 2.0 mg/kg (Table 2). The behavioral changes induced by the KA doses of 0.1 and 0.5 mg/kg were similar to those of PBS, thus it can be said that these two doses are subclinical.

Spontaneous scratching and WDS were also observed in the F1 1-BP-exposed group. The occurrence ratio of scratching was 100% at all doses of KA (Table 1), whereas that of WDS was 40 to 60% in 0 to 0.5 mg/kg and 100% in 2.0 mg/kg of KA (Tables 2). The effect of prenatal exposure to 1-BP was observed
in the occurrence ratio of WDS \((P < 0.01; \text{Mantel-Haenszel test})\). The occurrence ratio in the F1 1-BP-exposed group at 0.1 mg/kg KA was lower than that in the F1 control group \((P < 0.05 \text{ by Fisher’s exact test})\). The dose of 0.5 mg/kg KA tended to decrease the occurrence ratio in the F1 1-BP-exposed group, but did not reach a significant level. Taken together with the results of the 0.1 and 0.5 mg/kg KA (subclinical doses), the occurrence ratio (6 out of 12 rat pups) in the F1 1-BP-exposed group exhibited a lower value than that in the F1 control group (16 out of 16 rat pups, \(P < 0.005 \text{ by Fisher’s exact test}\)). This indicates that the effects of prenatal 1-BP exposure can be observed only at the subclinical doses of KA. The duration and the frequency of the scratching and the WDS increased at the dose of 2.0 mg/kg \((P < 0.01)\), but we did not find any significant effect of prenatal 1-BP exposure on the duration and frequency of WDS at any of the doses of KA. Our results suggest that prenatal exposure to 1-BP suppresses the occurrence of WDS only at a low dose of KA, possibly due to an effect on mechanisms underlying the generation of WDS.

WDS can be induced by electrical stimulation of limbic structures and by the administration of several chemicals, such as serotonergic compounds [15] and an opioid receptor agonist [16], as well as KA. KA-induced-WDS is depressed by μ-opioid receptor antagonists [16]. An antagonist of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/KA receptors suppresses WDS induced by serotonin receptor agonists [15]. The mechanisms of WDS induction by these chemicals are assumed to be related to each other. Besides those receptors, nitric oxide has also been demonstrated to play a regulatory role in KA- and gutt-wet-induced-WDS [9]. These receptors and nitric oxide might be the target of prenatal exposure to 1-BP.

### Table 1. The occurrence ratio, duration and frequency of scratching in F1 control and 1-BP-exposed groups

<table>
<thead>
<tr>
<th>KA (mg/kg)</th>
<th>S/N</th>
<th>duration (s)</th>
<th>frequency (counts)</th>
<th>S/N</th>
<th>duration (s)</th>
<th>frequency (counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>5/5</td>
<td>1.9 ± 0.3</td>
<td>21 ± 6</td>
<td>5/5</td>
<td>2.3 ± 0.3</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>0.1</td>
<td>11/11</td>
<td>2.0 ± 0.1</td>
<td>14 ± 2</td>
<td>7/7</td>
<td>2.0 ± 0.1</td>
<td>26 ± 10</td>
</tr>
<tr>
<td>0.5</td>
<td>5/5</td>
<td>1.7 ± 0.3</td>
<td>25 ± 4</td>
<td>5/5</td>
<td>2.1 ± 0.5</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>2.0</td>
<td>5/5</td>
<td>2.9* ± 0.4</td>
<td>557* ± 164</td>
<td>5/5</td>
<td>3.6* ± 0.2</td>
<td>517* ± 41</td>
</tr>
</tbody>
</table>

F: first filial generation, 1-BP: 1-bromopropane, KA: kainate, PBS: phosphate buffered saline, S: the number of rats in which scratching was observed, N: the total number of rats used in the experiment, S/N: the occurrence ratio, *: significant effects of KA on the duration or the frequency by two-way ANOVA followed by Scheffe’s test \((P < 0.01)\), mean ± SEM: mean ± standard error of the mean

### Table 2. The occurrence ratio, duration and frequency of WDS in F1 control and 1-BP-exposed groups

<table>
<thead>
<tr>
<th>KA (mg/kg)</th>
<th>S/N</th>
<th>duration (s)</th>
<th>frequency (counts)</th>
<th>S/N</th>
<th>duration (s)</th>
<th>frequency (counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>4/5</td>
<td>0.3 ± 0.02</td>
<td>1.8 ± 0.6</td>
<td>3/5</td>
<td>0.2 ± 0.03</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>0.1</td>
<td>11/11</td>
<td>0.3 ± 0.03</td>
<td>3.4 ± 0.6</td>
<td>4/7*</td>
<td>0.3 ± 0.09</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>0.5</td>
<td>5/5</td>
<td>0.3 ± 0.02</td>
<td>2.6 ± 0.9</td>
<td>2/5</td>
<td>0.2, 0.3</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>2.0</td>
<td>5/5</td>
<td>0.3 ± 0.05</td>
<td>29.2* ± 10.0</td>
<td>5/5</td>
<td>0.4 ± 0.02</td>
<td>54.4* ± 21.1</td>
</tr>
</tbody>
</table>

WDS: wet dog shakes, F: first filial generation, 1-BP: 1-bromopropane, KA: kainate, PBS: phosphate buffered saline, S: the number of rats in which WDS were observed, N: the total number of rats used in the experiment, S/N: the occurrence ratio, a: a significant difference between F1 control and F1 1-BP-exposed groups at the dose of 0.1 mg/kg in the Fisher’s exact test \((P < 0.05)\), *: significant effects of KA \((P < 0.01)\) on the two-way ANOVA followed by Scheffe’s test, mean ± SEM: mean ± standard error of the mean. The data of durations in the F1 1-BP-exposed group administered 0.5 mg/kg of KA are shown in the duration(s) column.
Developmental Neurotoxicity in Rats Prenatally Exposed to 1-BP

There are studies suggesting that the hippocampus is the target of KA. KA receptors have been found in the hippocampus in rat pups [12], and epileptic discharges have been observed when KA-induced seizures occur [17]. Moreover, KA-induced WDS was accompanied by robust electrographic seizures recorded from the hippocampus [18]. On the other hand, Fueta et al. have reported that prenatal 1-BP exposure decreases the paired-pulse ratio of population spikes in the CA1 subfield of the dorsal hippocampus in PND14 rats [19]. A decrease in the paired-pulse ratio of the population spike is generally interpreted as an increase in an inhibition [2]. Thus, prenatal 1-BP exposure may disturb the propagation of hyperactivity in the hippocampus, such as electrographic discharges associated with KA-induced WDS. This may account for the suppression of WDS by prenatal exposure to 1-BP. However, it should also be considered that the dentate gyrus (DG) in the ventral hippocampus is thought to be necessary for chemical interventions such as KA-, μ-opioid-, and electrical stimulation-induced WDSs in adult rats [16, 20, 21]. Therefore, further studies are needed to investigate the excitability of the DG in the ventral hippocampus in prenatally 1-BP-exposed rats.

In conclusion, we demonstrate here that prenatal exposure to 1-BP suppresses WDS induced by the administration of a low dose of KA. Our results indicate that prenatal 1-BP exposure may disturb the susceptibility to KA or the functions of neural networks related to the WDS. We also show that it may be advantageous to use pharmacological interventions with convulsants in investigations of the effects of environmental chemicals on behavioral responses in immature rats.

Conflict of Interest

No conflicts of interest to declare.

Acknowledgments

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References

1-プロモプロパンへの胎生期曝露は発達期ラットにおいてカイニン酸で誘導されるWet Dog Shakesを抑制する

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要 旨：1-プロモプロパン(1-BP)は洗浄やスプレー接着剤の溶剤として用いられている。1-BPの有害性はヒトの事例や成獣を用いた動物で報告されてきた。発達毒性も報告されてはいるが、発達神経毒性についての詳細はわかっていないうえ、我々は、1-BPの胎生期曝露が、発達期ラットへのカイニン酸投与により誘導される行動、すなわちscratching行動やwet dog shake様行動に及ぼす影響を調べた。ウィスター系妊娠ラットの妊娠1日目から20日目まで(6時間/日)、濃度700 ppmの1-BP蒸気を曝露出した。生後14日目の対照群と1-BP曝露群にカイニン酸を0.1、0.5、2.0 mg/kgで腹腔内投与した。Scratching行動に関しては対照群と1-BP曝露群に違いは見られなかったが、wet dog shake様行動に関しては、低濃度である0.1 mg/kgにおいて発生率の低下が1-BP曝露群で見られた。1-BP胎生期曝露が発達期の神経行動に影響することが示唆された。

キーワード：1-プロモプロパン、胎生期曝露、発達神経毒性、wet dog shake、ラット。

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