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Effects of sub-acute and sub-chronic inhalation of 1-bromopropane on neurogenesis in adult rats☆

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ABSTRACT

Purpose: 1-Bromopropane (1-BP) intoxication is associated with depression and cognitive and memory deficits. The present study tested the hypothesis that 1-BP suppresses neurogenesis in the dentate gyrus, which is involved in higher cerebral function, in adult rats.
Methods: Four groups of 12 male Wistar rats were exposed to 0, 400, 800, 1000 ppm 1-BP, 8 h/day for 7 days. Another four groups of six rats each were exposed to 0, 400, 800 and 1000 ppm 1-BP for 2 weeks followed by 0, 200, 400 and 800 ppm for another 2 weeks, respectively. Another four groups of six rats each were exposed to 0, 200, 400 and 800 ppm 1-BP for 4 weeks. Rats were injected with 5-bromo-2′-deoxy-uridine (*BrdU*) after 4-week exposure at 1000/800 ppm to examine neurogenesis in the dentate gyrus by immunostaining. We measured factors known to affect neurogenesis, including monoamine levels, and mRNA expression levels of brain-derived neurotrophic factor (BDNF) and glucocorticoid receptor (GR), in different brain regions.
Results: BrdU-positive cells were significantly lower in the 800/1000 ppm-4-week group than the control . 1-Week exposure to 1-BP at 800 and 1000 ppm significantly reduced noradrenalin level in the striatum. Four-week exposure at 800 ppm significantly decreased noradrenalin levels in the hippocampus, prefrontal cortex and striatum. 1-BP also reduced hippocampal BDNF and GR mRNA levels.
Conclusion: Long-term exposure to 1-BP decreased neurogenesis in the dentate gyrus. Downregulation of BDNF and GR mRNA expression and low hippocampal norepinephrine levels might contribute, at least in part, to the reduced neurogenesis.

1. Introduction

1-Bromopropane (1-BP) was introduced as an alternative to ozone-depleting solvents, such as chlorofluorocarbons and 1,1,1-trichloroethane, and is mainly used as a cleaning agent for metal parts of precision instruments and as a solvent in spray adhesives (Ichihara, 2005; Ichihara et al., 2002). 1-BP is also currently used in the United States in the dry cleaning industry. However, previous studies have shown that 1-BP is neurotoxic in humans and rats. Human cases of 1-BP toxicity showed ataxia (Ichihara et al., 2002; Raymond and Ford, 2007), sensory deficits (Ichihara et al., 2002; Majersik et al., 2007; Raymond and Ford, 2007; Sclar, 1999), hyperreflexia in lower extremities (Majersik et al., 2007; Raymond and Ford, 2007; Sclar, 1999), prolongation of distal latency in motor nerve (Sclar, 1999), and decrease in sensory nerve conduction velocity (Sclar, 1999). Apart from the above neurologic deficits, disorders of the higher cerebral function including depression, anxiety and memory deficits in humans were also reported (Ichihara et al., 2004, 2002; Majersik et al., 2007). These were also supported by experimental studies, which showed reduced noradrenalin axons in rat prefrontal cortex and amygdala (Mohideen et al., 2011) and neurobehavioral abnormalities (Honma et al., 2003) in rats after exposure to 1-BP.

The mechanism of disorders of the higher cerebral function following exposure to 1-BP remains elusive. Rats treated with irradiation showed inhibition of hippocampal neurogenesis,

Abbreviations: 1-BP, 1-bromopropane; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2′-deoxy-uridine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GR, glucocorticoid receptor; HIP, hippocampus; HVA, homovanillic acid; NE, norepinephrine; PFC, prefrontal cortex; STA, striatum.

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impairment of conditional rule learning and memory for specific events (Winocur et al., 2006) and learning that depends on increased hippocampal neurogenesis (Gould et al., 1999). These studies show neurogenesis plays an important role in learning and memory. In addition, a number of investigators favor the notion of involvement of neurogenesis in mood disorders. A previous study indicated that the behavioral effects of chronic antidepressants are mediated through stimulation of hippocampal neurogenesis (Santarelli et al., 2003). Transgenic adult mice with impaired hippocampal neurogenesis exhibited anxiety-related behaviors (Revest et al., 2009).

To clarify the relationship between 1-BP exposure and neurogenesis, we selected three variables, glucocorticoid receptor (GR), brain-derived neurotropic factor (BDNF) and a battery of neurotransmitters. Previous studies suggested that GR mediates the function of glucocorticoid, and glucocorticoid binding to GR can modulate the expression of various genes, including those related to neurogenesis (Webster et al., 2002). Another factor that affects neurogenesis is BDNF, a protein abundantly present in both the brain and plasma. BDNF is reported to increase neuronal survival *in vitro* (Memberg and Hall, 1995) and potentiate neuron differentiation in the adult hippocampus (Palmer et al., 1997). Also, disorders of neurotransmitter level are important pathoetiological factors in depression. In this regard, neurotransmitter release also regulates adult neurogenesis (Vaidya et al., 2007).

Based on the understanding of the relationships among disorders of higher cerebral function, neurogenesis, GR, BDNF and neurotransmitters, the present study was designed to investigate the effect of exposure to 1-BP on neurogenesis, the expression of BDNF and GR in the hippocampus, and monoamine levels in different brain regions, including hippocampus, in adult rats. Such information should enhance our understanding of the mechanism underlying disorders of higher cerebral function induced by exposure to 1-BP.

2. Materials and methods

The entire experiment was conducted according to the Guide of Animal Experimentation of Nagoya University, School of Medicine, concerning protection and control of animals.

2.1. Inhalation system

The inhalation exposure system was described in previous study (Ichihara et al., 2000). In brief, a regulated volume of 1-BP was evaporated at room temperature and mixed with a larger volume of clear air to achieve the target concentrations. The vapor concentration of 1-BP in the chamber was measured every 10 s by gas chromatography and electronically controlled to within ±5% of the target dose.

2.2. Animals and exposure to 1-BP

The study consisted of three parts. In the first experiment, 48 male Wistar rats purchased from Shizuoka Laboratory Center (SLC), Inc. Japan, were divided into four groups of 12 each and rats of each group were exposed to 1-BP at 0, 400, 800 and 1000 ppm using a custom-made inhalation system, at 8 h/day from 9:00 to 17:00 for 1 week. The age and body weight at the time of study entry were 10 weeks and 270–290 g, respectively. Six rats per group were used for biochemical studies and the remaining six rats were used for histopathological studies on neurogenesis.

In the second longer exposure experiment, 24 male Wistar rats (SLC, Inc. Japan) were divided into four groups of six rats each and exposed to 1-BP for 4 weeks to investigate the effect of longer exposure to 1-BP on neurogenesis. In these experiments, 1-BP concentration was set at 0, 400, 800 and 1000 ppm in the first 2 weeks, then reduced from 400 to 200, from 800 to 400, and from 1000 to 800 ppm in the second two weeks of the 4-week experiment because one rat died after 2-week exposure to 1000 ppm. Inhalation was conducted 8 h/day from 21:00 to 5:00 for 4 weeks in total. The age and body weight at study entry were 11 weeks and 320–350 g, respectively.

In the third longer exposure experiment, 24 rats were divided in four groups of six rats each and each rat was exposed to 1-BP at 0, 200, 400 and 800 ppm using the inhalation system 8 h/day from 21:00 to 5:00 for 4 weeks to study the effect of 4-week exposure to 1-BP on biochemical parameters. The age and body weight at study entry were 13 weeks and 330–360 g, respectively. Before exposure, the rats were acclimated in a light-controlled room [room temperature (23–25 °C), stable humidity (57–60%), 12 h light cycle starting at 9:00 am] and had free access to food and water.

The mean concentration of 1-BP measured every 10 s for 8 h was considered the value for that day. The daily time-weighted average of the concentration was 414 ± 12, 814 ± 21 and 1047 ± 23 ppm (n = 10, mean ± standard deviation) in the first experiment, and 379 ± 34, 756 ± 65 and 940 ± 90 ppm (n = 16) from the beginning to the 14–16th day of the experiment, and 199 ± 14, 386 ± 14 and 768 ± 25 ppm (n = 15) from the 15–17th day until the end of exposure. The time-weighted averages of exposure levels in the second part of the experiment (weeks 3 and 4) of the second experiment were 288 ± 7, 559 ± 13 and 851 ± 7 ppm, while those for the third experiment were 194 ± 12, 393 ± 13 and 775 ± 24 ppm (n = 31).

2.3. BrdU (5-bromo-2′-deoxy-uridine) injection

To determine the effect of 1-BP exposure on neurogenesis, after the last exposure, 12 rats of each group were each injected with BrdU (Sigma, St. Louis, MO) at 24 mg/100 g body weight in 0.9% saline, i.p. every 2 h for three times.

2.4. Dissection and tissue preparation

Rats injected with BrdU were transcardially perfused with 4% paraformaldehyde (PFA) 12 h after the last injection. The whole brain was dissected out and postfixed in 4% PFA for 24 h. Subsequently, the brain samples were placed into 10% sucrose solution overnight then placed into 20% sucrose solution overnight and later transferred into 30% sucrose solution. After overnight incubation in 30% sucrose solution, the brain samples were embedded in optimal cutting temperature (OCT) compound and frozen on dry ice. Frozen blocks were stored in −80 °C.

On the other hand, 12 rats of each group that were not injected with BrdU were decapitated and the whole brain was dissected out within 5 min. The hippocampus, striatum and prefrontal cortex were separated and frozen on dry ice immediately. The adrenal glands were cut removed and frozen on dry ice simultaneously. Blood samples were collected using heparinized funnel soon after decapitation.

2.5. Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

The hippocampal mRNA expression levels of BDNF and GR were measured by quantitative RT-PCR. Briefly, the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) was used to extract total RNA from the hippocampus. The RNA products were stored at −80 °C after extraction. SuperScript III Reverse Transcriptase Kit was used for mRNA reversal. After reverse-transcription, quantitative RT-PCR was performed using the M×3005P QPCR System (Agilent Technologies, Waldbronn, Germany). The primers and probes used in this reaction were designed by the Universal Probe Library (Roche Diagnostics): BDNF primers: forward, 5′AGCGCGAATGTGTTAGTGGT; reverse, 5′GCAATTGTTTGCCTCTTTTTCT, GR primers: forward, 5′GACATGGAAGCTGCAGAGTA; reverse, 5′TCGTTTCTTCCAGCATAAAGGT. Beta-actin was used as the control gene, using the primers: forward, 5′CCCGCGAGTACAACCTTCT; reverse, 5′CGTCATCCATGGCGAACT.

2.6. Measurement of serum levels of corticosterone and neurotransmitter levels in the hippocampus, striatum and pre-front cortex

Serum corticosterone levels were measured by a radioimmunoassay kit (Corticosterone EIA kit, Cayman Chemical, Ann Arbor, MI). The levels of monoamines, including noradrenalin, dopamine, serotonin, and their metabolites, were measured in the hippocampus, striatum and pre-front cortex (PFC), respectively, using high performance liquid chromatography (HPLC) with an electrochemical detector (ENO-10, Eicom Co., Kyoto, Japan).

In brief, the brains were quickly removed, and the hippocampus, striatum and PFC were dissected out on an ice-cold glass plate. Each brain region was rapidly frozen and stored at −80 °C until assayed. Each frozen brain sample was weighed and homogenized with an ultrasonic processor in 65 μl of 0.2 M perchloric acid containing isoproterenol as an internal standard. The homogenates were placed in ice for 30 min and centrifuged at 20,000 × g for 15 min at 4 °C. The supernatants were mixed with 1 M sodium acetate to adjust the pH to 3.0 and injected into an HPLC system equipped with a reversed-phase ODS column (Eicompak SC-5 ODS; 2.1 × 150 mm; Eicom) and an electrochemical detector. The column temperature was maintained at 25 °C, and the detector potential was set at +750 mV. The mobile phase was 0.1 M citric acid and 0.1 M sodium acetate, pH 3.5, containing 17% methanol, 190 mg/l sodium-L-octanesulfonate and 5 mg/l EDTA, and the flow rate was set at 0.23 ml/min. The turnover of monoamines was calculated from the content of each monoamine and their metabolites.

2.7. Immunohistochemistry and quantification of BrdU-positive cells

Coronal brain sections of 30 μm were cut on cryostat and mounted on slides. Every three sections were collected, thus covering the whole dentate gyrus (Bregma −2.56 to −6.04 mm). Sections were post-fixed in 4% PFA for 20 min, and treated with Nonidet P-40 (NP-40) overnight. They were later denatured with 5 M HCl for 20 min and then blocked with 10% goat serum for 1 h. BrdU-positive cells were labeled using