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Short Communication

Decreased Paired-pulse Inhibition in the Dentate Gyrus of the Brain in Rats Exposed to 1-Bromopropane Vapor

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1-bromopropane (1-BP) and its isomer 2-BP have been used as substitutes for chlorofluorocarbons. 1-BP has been suspected of having a more potent toxic effect on the nervous system than 2-BP[1]. In the peripheral nervous system, 1-BP reduced conduction velocity and increased the latency of the motor nerves, and degeneration of the myelin sheath has been observed in the rat[2, 3]. In the central nervous system, cell degeneration of Purkinje cells has been reported[2, 4], but no studies have focused on the effects of 1-BP on the neuronal function in the brain. We studied the effects of 1-BP inhalation on neuronal function and observed abnormal responses in the dentate gyrus (DG) of brain slices obtained from rats with convulsion induced by 1-BP inhalation in our preliminary study. We therfore became interested in functional changes involved in behavioral changes. The aim of this study was to investigate changes in neuronal excitability in the brain of rats subchronically exposed to 1-BP. Paired-pulse analysis has been widely used to study changes in hippocampal excitability in physiological[5] and pathological episodes[6–9]. In particular, the DG is thought to control excitatory inputs to the hippocampus from the cortex and it has been suggested that it is a determining factor in the spread of seizure activity in the hippocampus[10]. In the present study, paired-pulse population spike (PS) responses in the DG of rats which inhaled 1-BP were analyzed and compared with those of control rats. This is the first report describing the effects of 1-BP on neuronal function in the central nervous system.

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Materials and Methods

1-BP was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). More than thirty male Wistar rats (6 weeks old) were purchased from Kyudo Co., Ltd. (Japan) and were divided into control and exposure groups. The inhalation apparatus was as previously described[11]. The rats were placed in an inhalation chamber for 6 h a day for 5 d per week with a 1-BP concentration of 1,500 ppm for the exposure group and with only fresh air for the control group[4]. Slice preparations were done after 1, 3 and 4 wk of exposure, and after 1 wk of clearance following the 4 exposure wk. As previously reported by Ohnishi *et al.*[4], ataxic gait and convulsion were observed in some rats on the 5th day of the fourth week. Paired-pulse stimulation was applied to control (n=14) and 1-BP exposed (n=16) rats which did not have any abnormal behavior. Transverse hippocampal slices (450 μm in thickness) were prepared as previously described[8, 9]. They were transferred to an interface-type chamber and perfused with artificial cerebrospinal fluid (ACSF) saturated with a O_2/CO_2 mixture (95%: 5%) at a flow rate of 1 ml/min. The composition of ACSF in mM was: NaCl, 124; KCl, 2; KH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 2; $NaHCO_3$, 26; and glucose, 10. The experiments were performed under the Guiding Principles for the Care and Use of Animals approved by the Faculty Meeting of the University of Occupational and Environmental Health (UOEH).

We measured PSs for the synchronously firing neurons in adjoining cell layers in the DG. PSs were recorded from the granule cell layer in the DG by means of glass microelectrodes filled with 3M NaCl (1–2 MΩ). A bipolar stimulation electrode was placed on the lateral perforant path of the DG (Fig. 1A). We used the intensity of the stimulating current pulse to evoke barely maximal PS in order to induce the maximum number of excited cells. The PS amplitude was measured by projecting a line from the negative peak to a tangent line connecting the spike onset and offset (Fig. 1B). Calculation of the paired-pulse ratio (PPR) was done as follows: PPR of PS=2nd PS/1st PS. Interpulse intervals (IPIs) of the paired-pulse stimulation were 5, 10, 20, 50, 100, 200, 500 and 1,000 msec. Statistical significance was evaluated by unpaired Student's *t*-test to compare the 1-BP and control groups. The values represented the mean ± S.E.M.

Results and Discussion

Figure 1C shows typical examples of paired-pulse responses recorded from the DG of the 1-BP and control rats at 10 msec IPI. The control rats exhibited a strong depression of the second PS, while the 1-BP rats expressed almost complete PS in response to the second stimulation. The PPRs of 1-BP rats were consistently higher at the short IPIs of 5, 10 and 20 msec than those

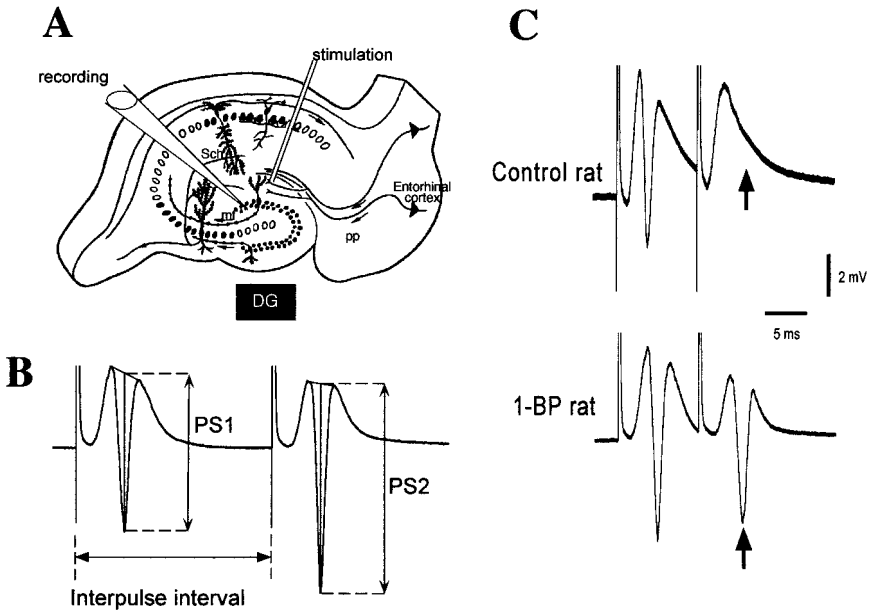

Fig. 1. Paired-pulse stimulation paradigm and typical examples from control and 1-bromopropane (1-BP)-exposed rats. A: A schema of hippocampal slice and position of stimulating and recording electrodes (see text for details). pp: perforant path connecting neurons of entorhinal cortex to dendrites of granule cells in the dentate gyrus. B: Conventional calculation of PS1 and PS2. PS: population spike C: Examples of paired-pulse PS responses. Paired-pulse responses were evoked by two pulses with a 10 msec interpulse interval. Note that the control rats had strong depression of the second PS (arrow in the upper trace), but the 1-BP rats had an almost complete PS in response to the second stimulation (arrow in the lower trace).

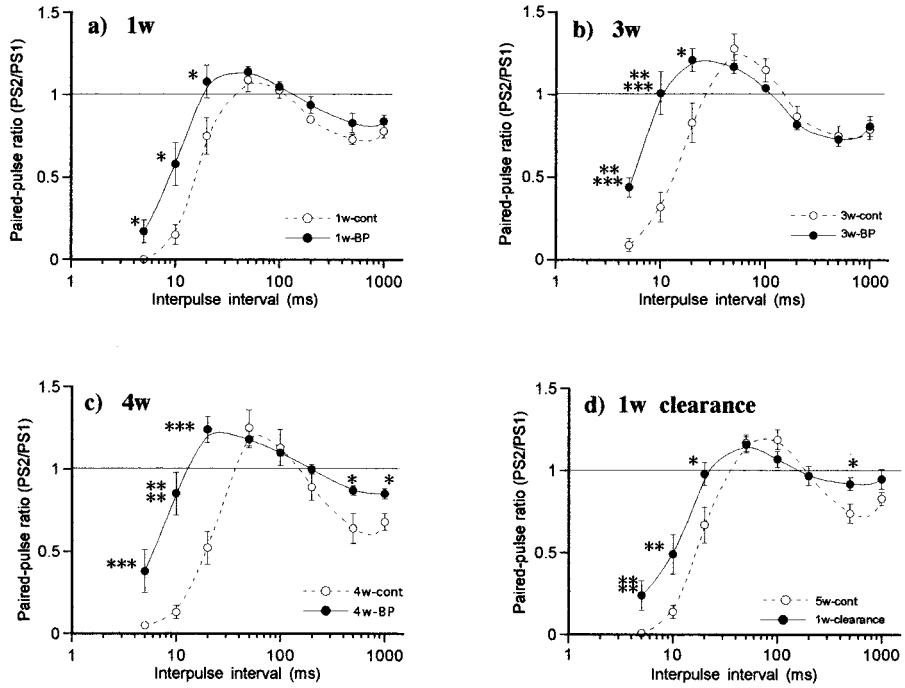

Fig. 2. Paired-pulse PS profiles after 1-wk (a), 3-wk (b), and 4-wk exposure (c), and 1-wk clearance after 4-wk exposure (d) to 1-bromopropane exposed (●) and control (○) rats. At short intervals (5, 10 and 20 msec), a decrease in paired-pulse inhibition was observed in 1 (a), 3 (b) and 4 (c) weeks of exposure. At longer interpulse intervals of 500 and 1,000 msec, the decrease was evident only at 4 wk of exposure. The decrease remained 1 wk after clearance with the exception of the paired-pulse ratio at 1,000 msec. (*: $P<0.05$, **: $P<0.01$, ***: $P<0.005$, ****: $P<0.001$, *****: $P<0.0005$, Student's *t*-test, unpaired). Values are the mean ± S.E.M. for 8–11 slices.

of control rats throughout the exposure period of 4 wk (Fig. 2a, b, and c). An increase in the PPRs of PSs in the short interpulse intervals (5–20 msec) has been understood to indicate a decrease in inhibition[8]. At the fourth week of inhalation, PPRs in the long IPIs of 500 and 1,000 msec also became higher than those of the control rats (Fig. 2c). The increase in the PPRs remained until the end of the 1-wk clearance (Fig. 2d).

Our results suggest the possibility that behavioral abnormalities observed in rats subchronically exposed to 1-BP are caused by functional changes in the central nervous system as well as in peripheral neurons. After 1 wk of inhalation of 1-BP in our study, we observed no changes in the organ histology of the liver, kidneys, spleen or lungs, with the exception of testes and in serum biochemistry but not in organ weight, with the exception of a slight increase in body weight. Paired-pulse analysis would therefore be a sensitive ways to examine neurotoxicity in rats exposed to 1-BP. 1-BP concentrations in urine diminished within 1 h after the cessation of inhalation, but the concentrations of bromine ions decayed slowly, with a half time of 4.7 d for blood and 5.0 d for urine after 3 wk of exposure.

In conclusion, 1-BP exposure at 1,500 ppm for 4 wk was demonstrated to be a cause of neuronal disfunction in the DG of the brain, which may be one of the predisposing neural mechanisms that precede abnormal behavior. Because it is not clear if the changes in paired-pulse ratios is a phenomenon specific to 1-BP, further studies on 1-BP's neurotoxicity after chronic exposure to low concentrations remain necessary.

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