Hyperexcitability of the Hippocampal CA1 and the Dentate Gyrus in Rats Subchronically Exposed to a Substitute for Chlorofluorocarbons, 1-Bromopropane Vapor

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Abstract: Hyperexcitability of the Hippocampal CA1 and the Dentate Gyrus in Rats Subchronically Exposed to a Substitute for Chlorofluorocarbons, 1-Bromopropane Vapor: Yukiko FUETA, *et al.* The First Department of Medical Technology, School of Health Sciences, University of Occupational and Environmental Health—To investigate the effects on the central nervous system of subchronic exposure to 1-bromopropane (1-BP), which is a substitute for chlorofluorocarbons, we measured the hippocampal excitability of 1-BP-treated rats electrophysiologically. Male Wistar rats were exposed to 1-BP for 6-h in a day in an exposure chamber (1,500 ppm) for 4 wk. After the 1-, 3- and 4-wk inhalation, field excitatory postsynaptic potentials (fEPSPs), population spikes (PSs), fEPSP/spike (E/S) curve, paired-pulse profiles of fEPSP slopes and PS amplitudes recorded from CA1 pyramidal neurons, and dentate granule cells of the hippocampal slice obtained from the rats were analyzed. In 1-BP treated rats, E/S potentiation and a lower subthreshold of PSs were observed in the dentate gyrus (DG) after the 3 and 4-wk inhalation. Paired-pulse inhibition was reduced at 5–50 ms in the CA1 and at 5–20 ms in the DG after all of the inhalation. These changes were not associated with paired-pulse inhibition of fEPSPs. In the DG, the paired-pulse inhibition at the short interpulse intervals in rats exposed to 1-BP was pronounced by an application of A type gamma aminobutyric acid (GABA) receptor agonist pentobarbital. Impaired paired-pulse inhibition of granule cells at the short interpulse intervals was recovered after the application of N-methyl-D-aspartate (NMDA) type glutamate receptor antagonist DL-2-

amino-5-phosphonopentanoic acid. Convulsing rats observed after the 4-wk inhalation exhibited multiple PSs in the DG, and their second component was abolished by an application of this antagonist. Inhalation of 1-BP increased the neuronal excitability in the hippocampal CA1 and the DG. The hyperexcitability of the granule cells in the DG was at least due to an over-activation of NMDA receptors. (*J Occup Health* 2002; 44: 156–165)

Key words: 1-Bromopropane inhalation, Neurotoxicology, Hyperexcitability, NMDA receptors, Hippocampal formation, Animal experiment

1-bromopropane (1-BP) and its isomer 2-BP have been used as substitutes for chlorofluorocarbons, and the toxicity of 2-BP has been investigated. 2-BP has been shown to cause severe reproductive and hematopoietic disorders in humans^{1,2}, and the Korean Ministry of Labor established the threshold limit of 1 ppm in the work place³. Similarly, the threshold limit value of 1ppm for 2-BP was suggested in Japan⁴. Although 1-BP is also suspected of being a toxic substance, there has not been enough evidence to confirm the suspicion, leaving it controversial. Severe behavioral abnormalities such as hindlimb paralysis and/or convulsion were observed⁵⁻⁸ and peripheral neurotoxic changes were reported in animals exposed to 1-BP^{5,7-9}, whereas such toxicity of 1-BP was not confirmed by other investigators¹⁰. Nevertheless, Scalar¹¹ recently reported central as well as peripheral neurotoxicity in the case of a male worker who complained of weakness in the lower extremities and right hand, numbness, dysphagia and urinary difficulties following a 2 month exposure to a solvent mainly composed of 1-BP. It is therefore a matter of urgency to investigate the neurotoxicity of 1-BP in the central nervous system.

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Fig. 1. Paired-pulse stimulation paradigm. **A:** A schema of a hippocampal slice and position of stimulating and recording electrodes (see text for details). pp: perforant path connecting neurons of the entorhinal cortex to dendrites of granule cells in the dentate gyrus; rec: recording electrode; stm: stimulating electrode. **B:** Conventional measurement of fEPSP slope and PS. fEPSP: field excitatory postsynaptic potential, PS: population spike. **C:** Measurements of fEPSP slope1, fEPSP slope2, PS1 and PS2.

The authors first demonstrated a decrease in the paired-pulse inhibition of population spikes (PS) evoked in the dentate gyrus (DG) of hippocampal slices prepared from rats exposed to 1-BP vapor¹². A paired-pulse stimulation paradigm provides an important indicator of neuronal excitability by studying how the response to a first stimulation influences the response to a second one, and has been widely used to study changes in hippocampal excitability in physiological¹³ and pathological episodes^{14–17} (Fig. 1). The DG is thought to control excitatory inputs into the hippocampus from the cortex and it is suggested that it is a determining factor in the spread of seizure activity in the hippocampus¹⁸, so that the impairment of paired-pulse inhibition in the DG might contribute to the underlying mechanism that makes the hippocampus become hyperexcitable. In the present study, therefore, we extended our detailed analysis of hippocampal excitability to another area, CA1, which is known to have output connections to other areas of the brain, including the neocortex. For analysis, changes in the excitability of the hippocampal neurons were recorded

by monitoring the slope of the field excitatory postsynaptic potential (fEPSP) that represents the excitatory synaptic drive, and the amplitude of PS that reflects the synchronous discharge of a population of neurons (Fig. 1B). Thus it is possible to draw input-output relationships, so-called fEPSP/spike curves (E/S curves)¹⁹, by plotting the amplitude of PS as a function of the slope of fEPSP for various stimulus intensities. In this paper, the E/S curve and the paired-pulse responses of the fEPSP slope and PS amplitude evoked in the hippocampal CA1 and the DG of rats subchronically exposed to 1-BP were analyzed and compared with those of control rats.

Materials and Methods

Animals

Male Wistar rats (6 wk of age) were purchased from Kyudo Co., Ltd. (Japan). The rats were bred under the conditions of a 12-h light-dark cycle, controlled temperature ($24 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$), and free watering and -feeding. The experiments were performed

under the control of the Ethics Committee of Animal Care and Experimentation in accordance with The Guiding Principle for Animal Care Experimentation, the University of Occupational and Environmental Health, Japan and the Japanese Law for Animal Welfare and Care (No.221). The rats were divided into control (n=22) and exposure (n=25) groups. Each group was further separated into 3 groups: 1- (n=6), 3- (n=6) and 4- (n=10 for control and n=13 for exposure) wk inhalation.

Inhalation

The apparatus of the inhalation system was as previously described²⁰. 1-BP was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). The rats were placed in an inhalation chamber at a 1-BP concentration of 1,500 ppm for the exposure group and with only fresh air for the control group. These exposures were performed for 6 h a day, between 0900 h and 1500 h, for 5 d a week.

Hippocampal slice preparation and chemicals

The rats were subjected to electrophysiological procedures at the end of the 1st, 3rd and 4th wks of exposure. At the 4th wk of exposure, the rats that had ataxic gait and convulsions underwent a slice preparation procedure immediately after these abnormal forms of behavior were observed. After the last exposure in each experimental week, the rats were deeply anesthetized with diethyl ether and then decapitated. The brain was gently removed and dipped in cooled artificial cerebrospinal fluid (ACSF) saturated with an O₂/CO₂ mixture (95%: 5%). The composition of the ACSF in mM was: NaCl, 124; KCl, 2; KH₂PO₄, 1.25; CaCl₂, 2; MgSO₄, 2; NaHCO₃, 26; and glucose, 10. The hippocampus was quickly separated from other brain regions in the cool stage while being moistened with a cooled ACSF. Then transverse slices 450- μ m thick were obtained from the middle third region of the bilateral hippocampi with a McIlwain tissue chopper. The slice preparation was made within 2 h after inhalation. The slices were transferred to an interface-type recording chamber, which was controlled at 32 $\pm$ 0.5°C, and perfused with ACSF saturated with a mixture of O₂/CO₂ (95%: 5%) at a flow rate of 1 ml/min. DL-2-amino-5-phosphonopentameric acid (AP5, 50 μ M, Tocris), competitive antagonist of N-methyl-D-aspartate (NMDA) type glutamate receptor, and pentobarbital (PB, 100 μ M, Sigma), an enhancer of A type receptor for gamma aminobutyric acid (GABA), were made in stock solution. They were diluted with ACSF and perfused to the slices.

Stimulation and recordings

At 1-h stabilizing after slicing, recording glass microelectrodes (1–2 M Ω) were placed in the cell layer in CA1 or DG. Bipolar stimulation electrodes made of stainless wires (50 μ m in diameter) were placed on the

Schaffer collateral/commissural fibers for CA1 recording or on the perforant path for DG recording (Fig. 1A). Stimulations consisted of square-wave pulses from a stimulator (Nihon Kodens, SEN7203, Japan) via an isolator (Nihon Kodens, SS202J, Japan). The duration of the stimulating current pulse was fixed at 100 μ s. To study the E/S curve, single stimulation was applied and the intensity was increased from 10 to 1000 μ A about every 10 μ A in CA1 and about every 50–100 μ A in DG. In the paired-pulse configuration, the depression/potential in the responses to the second stimulation relative to those in the first stimulation is referred to as paired-pulse inhibition/facilitation, the intensity of which depends on interpulse intervals (IPIs) and stimulation intensity. In the present study the current amplitude was adjusted so as to give a barely maximum population spike, and the stimulation amplitude in which stable maximum responses were obtained was used throughout the experiments for paired-pulse analysis. IPIs of the paired-pulse stimulation were 5, 10, 20, 50, 100, 200, 500 and 1,000 ms. Electrophysiological signals were amplified with a high-impedance amplifier (Axon Instrument Co., Axoclamp 2B, U.S.A.) with a bandpass of 10 kHz. The signals were then digitized with an AD converter (Axon Instrument Co., Digidata 1200, U.S.A.) and stored in a personal computer with P-clamp software (Axon Instrument Co., U.S.A.). Finally, stored signals were displayed on a printer and/or were analyzed with Axograph software (Axon Instrument Co., U.S.A.) for detailed analysis.

Analysis

The slope of fEPSP was calculated as the amplitude change at 20–80% of the voltage difference between the start of the waveform and the fEPSP amplitude at the onset of PS (Fig. 1C, upper trace). The PS amplitude was measured by projecting a line from the negative peak to a tangent line connecting the spike onset and offset (Fig. 1C, lower trace). In the convulsive rats, the 1st component of multiple PS was taken as the PS amplitude.

To study the E/S curve, each value in the fEPSP slope and the PS amplitude of each trace was normalized by dividing by the maximal value in the fEPSP slope and the PS in each slice, to exclude aberration in the electrode position. Then the relative amplitude of PS was plotted as a function of the relative slope of fEPSP evoked by various stimulus intensities.

To analyse of paired-pulse responses, calculation of the paired-pulse ratio (PPR) was done as follows:

$$\begin{aligned}\text{PPR of fEPSP} &= \text{2nd fEPSP slope} / \text{1st fEPSP slope.} \\ \text{PPR of PS} &= \text{2nd PS} / \text{1st PS}\end{aligned}$$

Statistical significance was evaluated by the Mann-Whitney U test or the Student's *t*-test for the difference between the 1-BP and control groups. The paired *t*-test was used to examine the difference between the absence

Fig. 2. fEPSP/spike (E/S) curve in the hippocampal CA1 and the dentate gyrus (DG). Each value was normalized by maximal fEPSP and PS, respectively, in 1-bromopropane (1-BP) exposed (●) and control (○) rats. E/S potentiation was observed in the DG at the 3rd and 4th wks of inhalation. Abbreviations: fEPSP, field excitatory postsynaptic potentials; PS, population spike.

Table 1. Changes in the subthreshold of population spikes in CA1 and the dentate gyrus (DG). A subthreshold was expressed as the % of max fEPSP slope that was calculated for Fig. 1

Inhalation period	1w		3w		4w		
Age	7 wk-old		9 wk-old		10-wk old		
Group	control	1-BP exposed	control	1-BP exposed	control	1-BP exposed	
						No convulsion	convulsion
Subthreshold in CA1	19.8 ± 12.9 (n=6)	15.2 ± 10.8 (n=7)	10.3 ± 3.6 (n=4)	12.6 ± 11.2 (n=4)	14.2 ± 5.6 (n=5)	13.0 ± 4.1 (n=9)	–
Subthreshold in DG	48.7 ± 15.9 (n=5)	40.5 ± 15.7 (n=8)	40.2 ± 14.3 (n=8)	24.5 ± 12.1* (n=8)	43.7 ± 14.6 (n=9)	29.6 ± 15.6 (n=9)	23.4 ± 13.7* (n=9)

The data are the mean ± standard deviation. Abbreviations: fEPSP, field excitatory postsynaptic potential; 1-BP, 1-bromopropane.

* $p < 0.05$ compared to each control group by Mann-Whitney U-test.

and presence of the pharmacological manipulation.

Results

Behavior

In our inhalation condition, 8 out of 15 1-BP exposed rats suddenly manifested ataxic gait and convulsions at the end of the 4th wk after cessation of inhalation. Interestingly, the abnormal behavior was not observed during inhalation, but the day after the last inhalation day. The behavior was similar to that in previous studies^{5, 6}.

Responses to a single stimulation

The fEPSP slopes and PS amplitudes increased according to an increase in stimulation intensity, as shown in Figure 2. In the CA1, there was no change in the E/S curve or the average of the subthreshold for PS for 1-BP exposed and control rats (Fig. 2, upper panels, Table 1). In the DG, on the other hand, the E/S curve tended to shift to the left at the 3 and 4-wk inhalation, and the average of the subthresholds for PS was lower by 30–40% than for the control rats (Fig. 2, lower panels, Table

A

B

Fig. 3. Hyperexcitable responses evoked in the dentate gyrus obtained from 1-bromopropane (1-BP) rats with convulsions. A: fEPSP/spike potentiation. B: The second potential (arrow) of multiple population spikes almost disappeared after perfusion of N-methyl-D-aspartate (NMDA) type glutamate receptor antagonist DL-2-amino-5-phosphonopentameric acid (AP5). The effect of AP5 was restored after washing out. Abbreviations: fEPSP, field excitatory postsynaptic potentials; PS, population spike.

1). There was hardly any synchronized fEPSP or PS recorded from the CA1 of the 1-BP exposed rats with convulsions. In the DG of 1-BP exposed rats with convulsions, the E/S curve clearly shifted to the left (Fig. 3A) and the average of the subthresholds for PS was lower by 46% than in the control rats, and was similar to that of rats exposed to 1-BP for 3 wk (Table 1). There was a single PS evoked in CA1 and DG of slices obtained from control and 1-BP exposed rats, but multiple PSs were

recorded in the DG of rats with convulsions (Fig. 3B, upper trace) ($n=8$). The 2nd peak (arrow) of the multiple PSs was abolished in the presence of a competitive NMDA type glutamic acid receptor antagonist AP5 ($50 \mu\text{M}$), suggesting an over-activation of the NMDA receptors in the multiple PSs. The effect of AP5 was washed out with ACSF.

Maximum fEPSP and PS

Since the degree of PPR can be considerably altered according to the stimulation intensity, we chose a maximum stimulation intensity, by which maximally synchronized PS was evoked with the 1st stimulation^{16, 17}. Comparisons of a maximum stimulation, a maximum fEPSP slope, and a maximum PS between 1-BP exposed and control rats are summarized in Table 2. In CA1 and DG, there were no significant differences in the stimulation intensity to evoke maximum PS, the maximum fEPSP slope or the maximum PS for 1-BP exposed and control rats, with the exception of the maximum PS at the 1-wk inhalation in CA1, and the fEPSP slope at the 1-wk inhalation, and the stimulation intensity of the rats with no convulsion at the 4-wk inhalation in DG (Table 2). The three parameters therefore did not seem to be changed in proportion to the period of subchronic inhalation of 1-BP.

Paired-pulse analysis

Figure 4 shows a typical example of paired-pulse responses observed in the CA1 and DG areas of the control and 1-BP exposed rats. The control rats had strong paired-pulse inhibition, but almost maximum PS (arrow in the lower trace) was evoked with the second stimulation in 1-BP exposed rats, indicating an increase in the PPR in the 1-BP exposed rats.

Differences in paired-pulse profiles of the fEPSP slope and PS in control and 1-BP exposed rats were compared (Figs. 5 and 6). The increase in PPRs of PS at 5–50 ms IPIs was consistently observed in CA1 through the experimental periods of 1-, 3- and 4-wk inhalation. Changes in PPRs in the fEPSP slope were observed only at 5 ms IPI at the 1-wk inhalation and 50 ms at the 4-wk inhalation. As in the CA1, the increase in PPRs of PS was consistently observed at 5–20 ms IPIs in the DG at the 1-, 3- and 4-wk inhalations. Changes in PPRs in the fEPSP slope were observed only at 10 ms IPI at the 4-wk inhalation. Thus, the dissociation of changes in profiles of PSs with fEPSP slopes was observed in both the CA1 and the DG.

In order to study the involvement of inhibitory and excitatory neurotransmitter receptors in a decrease in paired-pulse inhibition, the effects of a NMDA type glutamate receptor antagonist AP5 ($50 \mu\text{M}$) were first studied, since activation of NMDA receptors was involved in multiple PSs evoked in the DG of slices obtained from

Table 2. Comparisons of a maximum stimulation, a maximum fEPSP slope and maximumPS in CA1 and the dentate gyrus (DG) in 1-BP exposed and control rats

Inhalation period Age	1w 7 wk-old		3w 9 wk-old		4w 10-wk old		
CA1	control	1-BP exposed	control	1-BP exposed	control	1-BP exposed	
						No convulsion	convulsion
	n=6	n=7	n=5	n=6	n=6	n=5	n=3
Maximum stimulation [μ A]	74.3 $\pm$ 22.9	117.7 $\pm$ 58.8	169.6 $\pm$ 110.6	124.0 $\pm$ 104.0	97.5 $\pm$ 61.7	105.0 $\pm$ 12.0	–
Maximum fEPSP slope [mV/ms]	5.10 $\pm$ 1.65	4.25 $\pm$ 1.19	6.60 $\pm$ 2.15	4.57 $\pm$ 1.76	5.12 $\pm$ 1.66	4.10 $\pm$ 1.39	–
Maximum PS [mV]	28.4 $\pm$ 4.0	14.6 $\pm$ 5.1**	20.1 $\pm$ 4.9	16.7 $\pm$ 7.8	17.1 $\pm$ 9.2	16.6 $\pm$ 3.8	–
DG	control	1-BP exposed	control	1-BP exposed	control	1-BP exposed	
						No convulsion	convulsion
	n=5	n=8	n=11	n=8	n=7	n=14	n=9
Maximum stimulation [μ A]	478.0 $\pm$ 109.0	576.3 $\pm$ 154.3	540.5 $\pm$ 219.5	668.8 $\pm$ 104.7	832.9 $\pm$ 193.4	546.4 $\pm$ 194.8**	711.1 $\pm$ 208.8
Maximum fEPSP slope [mV/ms]	6.84 $\pm$ 1.38	3.55 $\pm$ 1.01**	2.87 $\pm$ 2.13	2.74 $\pm$ 0.88	3.67 $\pm$ 2.69	3.19 $\pm$ 0.95	4.20 $\pm$ 2.07
Maximum PS [mV]	10.0 $\pm$ 2.1	7.9 $\pm$ 1.6	5.8 $\pm$ 2.4	6.9 $\pm$ 1.7	6.2 $\pm$ 3.7	7.4 $\pm$ 2.3	5.9 $\pm$ 3.5

The data are the mean $\pm$ standard deviation. Abbreviations: fEPSP, field excitatory postsynaptic potential; PS, population spike; 1-BP, 1-bromopropane. ** $p < 0.01$ compared to each control group by Mann-Whitney U test.

convulsing rats (Fig. 3). AP5 decreased PPRs at the short IPIs of 5 and 10 ms and the longer IPIs of 200, 500 and 1,000 ms (Fig. 7A). GABA_A receptor enhancer PB was independently applied to slices from 1-BP exposed rats. PB decreased PPRs in all intervals, suggesting less firing of granule cells in response to the second stimulation due to enhancement of inhibitory postsynaptic potentials/currents by PB (Fig. 7B). Therefore, an increase in GABA receptor activation and a decrease in NMDA receptor activation decreased the paired-pulse ratio of PSs.

Discussion

1-BP inhalation was shown to be neurotoxic and to induce hyperexcitability in the CA1 and DG. 1-BP induced behavioral abnormalities such as ataxic gait and convulsion, observed in the present study, were consistent with a previous study showing that hindlimbs became paralyzed and that the rats were seriously emaciated after 5 or 7 wk, even at a concentration of 1,000 ppm⁵. A reduction in motor nerve conduction velocity and increased motor latency in rat-tail nerve were observed in both inhalation^{5, 7, 8, 21} and injection⁹ of 1-BP. Changes in excitability in hippocampal neurons seem to occur earlier than damage to peripheral nerves, although our inhalation time schedule was slightly different from that used in other studies^{5, 7, 8, 21}. Decrease in paired-pulse inhibition was observed in both the hippocampal CA1

and the DG at the 1-wk inhalation, and persisted until the 4-wk inhalation. The decrease in paired-pulse inhibition of PSs was not associated with consistent changes in PPRs of the fEPSP slope in either CA1 or DG, suggesting the involvement of a reduction in recurrent inhibition rather than changes in excitatory drives to principal neurons¹⁷. Nevertheless, cellular effects of 1-BP on granule cells might differ from those on CA1 pyramidal cells, since lowering the subthreshold of PS and E/S potentiation was predominantly observed in DG, but it was hardly observed in CA1. The difference in the maximum PS in CA1 and the difference in the maximum fEPSP slope in DG at the 1-wk inhalation might indicate that 1-BP inhalation could lead to more deleterious effects on younger rats. The increase in body weight was significantly reduced after 1w-inhalation in rats in the experimental group (see results and discussion of reference No.12). The change in body weight seems to have no bearing on the changes in paired-pulse inhibition, because 700 ppm of 1-BP also decreased in paired-pulse inhibition in the hippocampus with no change in body weight in rats in the experimental groups (unpublished data).

In our preliminary study, paired-pulse inhibition was completely diminished in the DG obtained from rats with convulsions. Decrease in paired-pulse inhibition or recordings of multiple PSs in the hippocampal formation

Fig. 4. Example of paired-pulse PS responses at the 1st wk of 1-bromopropane (1-BP) inhalation. Note that the control rats had strong depression of the second PS, but the 1-BP rats had an almost complete PS in response to the second stimulation (arrow in the lower trace) in both CA1 and the dentate gyrus (DG). Abbreviation: PS, population spike.

have been reported in some cases of brain tissue surgically removed from patients with epilepsy²²), of genetic models of epilepsy¹⁶) and of chemical or stimulation induced epilepsy in animals^{15, 23}). Decrease in paired-pulse inhibition has been observed in the hippocampal formation of animals exposed to other volatile organic solvents in our preliminary experiments (unpublished data).

From the results of AP5 application, over-activation of NMDA receptors was involved in both the decrease in paired-pulse inhibition and the multiple PSs in the DG. These findings could indicate that the changes in excitability in the central nervous system quite precede the behavioral changes, including convulsion.

Br⁻ itself seems to be an anticonvulsant, as it was reported that sodium bromide (5–7 mM) increased paired-

pulse inhibition and blocked seizure-like events in epilepsy models in brain slices²⁴). The neurotoxic action of 1-BP may not be direct but through intermediate metabolites. Propylene oxide, one of the intermediate metabolites of 1-BP, caused ataxia in the hindlegs and axonal degeneration in the hindleg nerves and in the fasciculus gracilis²⁶). Because the metabolic pathway of 1-BP was complicated²⁷), the causal chemical substance was not determined in this study. Disinhibition at the short IPIs in the CA1 and DG might be caused by decreased GABAergic recurrent inhibition^{16, 17}). Since GABA is a major inhibitory neurotransmitter, the reduction in the GABA-mediated postsynaptic hyperpolarizing potentials could easily lead postsynaptic potentials to depolarization. In fact, a decrease in GABAergic inhibition has been suggested as a powerful

Fig. 5. Paired-pulse profiles of field excitatory postsynaptic potential (fEPSP) and population spike (PS) in the CA1 after 1-, 3- and 4-wk exposure to 1-bromopropane (1-BP) exposed (●) and control (○) rats. A decrease in paired-pulse inhibition of PS was observed at short intervals (5–50 ms). There were no consistent changes in paired-pulse ratios of the fEPSP slope in 1-BP and control rats. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, Student's t -test). Values are the mean $\pm$ standard error for 6–8 slices.

Fig. 6. Paired-pulse profiles of field excitatory postsynaptic potential (fEPSP) and population spike (PS) in the dentate gyrus after 1-, 3- and 4-wk exposure to 1-bromopropane (1-BP) (●) and control (○) rats. A decrease in paired-pulse inhibition of PS was observed at short intervals (5–20 ms). At longer interpulse intervals of 500 and 1,000 ms, the decrease was evident only at 4 wk of exposure. There were no consistent changes in paired-pulse ratios of fEPSP slope in 1-BP and control rats. (*: $p < 0.05$, ***: $p < 0.001$, Student's t -test). Values are the mean $\pm$ standard error for 8–11 slices.

Fig. 7. Involvement of receptors for excitatory (A) and inhibitory (B) synaptic transmittance in the decrease in paired-pulse inhibition observed in the dentate gyrus of rats exposed to 1-bromopropane (1-BP). A: N-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonopentametric acid (AP5) decreased the paired-pulse ratios at the short interpulse intervals of 5 and 10 ms and at the longer interpulse intervals of 200–1,000 ms in 1-BP rats. B: Pentobarbital (PB), enhancer of A type gamma aminobutyric acid receptor, increased the paired-pulse inhibition in most overall ranges. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ by paired t -test. Abbreviation: PS, population spike.

hypothesis explaining hyperexcitable pathology epilepsy²⁷⁾. The depolarization of the membrane potential may release Mg^{2+} blocking of NMDA receptor activation and result in activation of NMDA receptors²⁸⁾. It is suggested that changes in GABA inhibition change the E/S curve²⁹⁾. Accordingly, at least granular neurons of the DG would become hyperexcitable, leading to convulsions associated with multiple PSs in the DG. This hypothesis could not be applicable to the CA1, as there were no changes in the E/S curve in the CA1 and hardly any synchronized potentials were evoked in the CA1 area obtained from convulsing rats. Pyknotic changes were extended to almost the whole CA1 area in those rats compared to control rats (data not shown). Therefore, failure in recording evoked potentials may be due to the occurrence of cell damage in the CA1. Since CA1 pyramidal neurons are thought to be vulnerable to anoxic or ischemic conditions, the histological changes would presumably result from convulsions.

Among brain regions, the hippocampus has been thought to play an important role in episodic memory and learning^{30–32)}. For that reason, hippocampal slices have been used for studying synaptic plasticity such as long-term potentiation (LTP), which is thought to be a basic physiological mechanism underlying memory and learning in rats. LTP was associated with an E/S potentiation, when the E/S curves were shifted to the left¹⁹⁾. To date, hippocampal slice techniques have not been very popular for studying neurotoxicity induced by industrial chemicals, but it would be a useful tool for studying functional changes with test batteries of excitatory and inhibitory changes, and plasticity. In

accordance with this, Ogawa³³⁾ recommended brain slice techniques as one of the screening tests of neurotoxicity in the brain induced by industrial chemicals.

In conclusion, subchronic exposure to 1-BP in rats caused changes in excitability, such as over-activation of NMDA receptors of the granule cells of the DG. Furthermore, activation of NMDA receptors is prerequisite for induction of some type of LTP^{34, 35)}; therefore the effects of 1-BP on LTP induction and maintenance should continue be studied.

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