Short Communication

ALTERATION OF CATECHOLAMINE
METABOLISM IN RAT BRAIN
PRODUCED BY INHALATION
EXPOSURE TO METHYL BROMIDE臭化メチル吸入暴露によるラット脳内
カテコラミン代謝の変化

Key words: methyl bromide; norepinephrine; 3-methoxy-4-hydroxyphenylglycol; tyrosine hydroxylase; DOPA; HPLC; native fluorescence.

Methyl bromide (CH_3Br ; MB) exerts a neurotoxic effects on human beings¹⁾ and experimental animals.^{2,3)} To evaluate the toxicity of MB, we exposed rats to MB gas and investigated the neurochemical changes in discrete brain areas.^{4,5)} Microanalysis of monoamines demonstrated changes in brain levels of catecholamine and metabolites.⁵⁾ In our previous study,⁸⁾ an 8-h exposure of rats to 125 ppm MB produced a drop of body temperature and inhibition of food intake. Hyperthermia and anorexia were occasionally observed in MB intoxicated patients. Nerve cells which regulate body temperature and appetite are known to be located in hypothalamus. In the present study, we investigated MB-induced changes of catecholamine metabolism in hypothalamus and mode of inhibition of tyrosine hydroxylase (TH) activity in rat brain following a single exposure. A new assay system was introduced for measurement of TH activity.

Methods. Groups of male SD rats (Charles River Japan), 250 to 300 g in body weight, were exposed to MB gas for 8 h from 6:00 a.m. Procedures for exposure have been described previously.⁴⁾ Following the exposure, rat head used for measurement of catecholamine contents was irradiated by microwave power (4.6 kW, 1.9 s) and decapitated. Rat subjected to estimation of TH activity was killed by decapitation. Brain was dissected and stored at -80°C .⁴⁾ Procedures of quantitation of catecholamine and metabolite with HPLC have been described previously.⁶⁾ TH activity was measured as follows. Hypothalamus was homogenized in Tris buffer, pH 7.25, containing sucrose and Triton X-100. Reaction medium was mixed with substrate, L-tyrosine (D-tyrosine for control) solution. The reaction medium contained 6-methyl-5,6,7,8-tetra-

hydropterine (1.2 mM in final concentration), mercaptoethanol (final 90 mM), catalase 300 units, NSD-1015 (decarboxylase inhibitor, final 100 μM), and acetate buffer, pH 6.1 (final 0.4 M). The reaction was started by adding homogenate. Following incubation for 20 min at 37°C , reaction was stopped by adding HClO_4 solution. Addition of EDTA, acetate buffer, pH 6.1, and α -methyl DOPA (internal standard) was followed by K_2CO_3 solution to adjust pH to 5–7. The solution was freeze-dried overnight and centrifuged. DOPA in the supernatant formed by the enzyme reaction was extracted and purified by passing through Amberlite CG-50 column and adsorption on alumina. DOPA was liberated from alumina with HCl solution. HPLC procedures have been previously described.⁶⁾ Mobile phase used was $\text{Na}_2\text{-HPO}_4$ -citrate buffer, pH 4.00, containing EDTA, 1-octanesulfonate, and methanol. DOPA and α -methyl-DOPA were detected by measuring native fluorescence.⁶⁾

Results and discussion. In the present new detection system of DOPA, a linear relationship was observed between fluorescence intensity and amounts of DOPA (and α -methyl DOPA) ranging widely from 1 to 800 pmoles. In a previous assay procedure, DOPA was detected by an electrochemical detector. The sensitivity of the present assay system was comparable to the electrochemical detector.⁶⁾ Rats were exposed to 16, 31, 63, 125, 188, or 250 ppm MB for 8 h and sacrificed immediately after exposure.

NE in hypothalamus was reduced by MB exposure in an MB concentration dependent manner (Fig. 1). Exposure at 16 ppm produced no significant change in NE content (data not shown). Percent decrease compared to control was 13% at 31 ppm and 30% at 250 ppm. NE decrease after 8-h exposure at 250 ppm was similar to that after 24-h exposure at 100 ppm (29%) as reported previously.⁴⁾ 3-Methoxy-4-hydroxyphenylglycol (MHPG), metabolite of NE, was increased by exposure. Percent increase compared to control was 34% at 250 ppm.

TH catalyzes hydroxylation of tyrosine to DOPA. NE, a principal neurotransmitter in the brain, is synthesized from tyrosine via DOPA and dopamine. Synthesis of DOPA is a rate limiting step in these serial reactions. Hypothalamus is known to be an NE neuron-rich region in the brain. TH activity of tissue homogenate was

Fig. 1. Effects of MB on NE and MHPG levels in rat hypothalamus. Mean $\pm$ SE ($n=5$). x: $p<0.05$, xx: $p<0.01$ by t -test. uv: Variances of control and exposed groups were unequal. Statistical significance was not observed by Aspin-Welch method.

Fig. 2. Lineweaver-Burk plots of TH activity of hypothalamus homogenate of rats exposed to MB. TH activity was determined in triplicate in each point. $\circ$: control, Δ : 63 ppm, $\square$: 250 ppm.

measured at substrate concentrations of 18, 35, and 100 μ M. At each substrate concentration, TH activity of exposed rat was lowered. From the Lineweaver-Burk plots, inhibition of TH activity by MB seems to be a mixed type of non-competitive and uncompetitive inhibition (Fig. 2). Maximum velocity and Michaelis constant obtained at each exposure concentration are shown in Table 1. V_m and K_m values at 250 ppm were 54% and 67%, respectively, compared with the control. These data suggest that a conformational change or a modification of enzyme molecule have occurred in TH of hypothalamus by MB exposure. V_m and K_m values at 16 ppm were similar to control values. At 31 ppm, however, apparent reduction in these parameters was observed. NE reduction at 31 ppm MB was statistically significant. Lowered NE level in hypothalamus seems to be elicited, at least partly, by the inhibition of TH.

Table 1. Maximum velocity (V_m) and Michaelis constant (K_m) of TH activity of hypothalamus homogenate.

MB concentration (ppm)	0	16	31	63	125	250
V_m (pmol/mg protein/min)	9.4	9.3	5.8	6.3	5.9	5.1
K_m (mM)	0.055	0.064	0.040	0.042	0.049	0.037

Increase of MHPG by MB exposure may be the results of inhibition of MHPG elimination from synaptic areas.

Inhibition of NE synthesis (and fall in activity of NE neuron) in hypothalamus is likely to be the background of the changes of body temperature and appetite observed in clinical cases and experimental animals. More attention should be paid to nervous functions which are deeply related to hypothalamus, e.g. autonomic nervous system, in MB toxicity.

In this study, we observed an efficacy of kinetic analysis in toxicity evaluation. Further detailed study of alteration of catecholamine metabolism should be made to investigate the neurotoxicity of MB.

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