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The Alterations in Neurotransmitters and Their Metabolites in Discrete Brain Regions in the Rats after Inhalation of Disinfectant, Glutaraldehyde or *ortho*-phthalaldehyde for 4 Weeks

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Abstract: Glutaraldehyde (GA) and *ortho*-phtalaldehyde (OPA) have been widely used as major components of disinfectants in hospitals. We evaluated the alterations in GA or OPA in rats after subacute inhalation exposure by determining levels of neurotransmitters (norepinephrine [NE], dopamine [DA], DA metabolites, dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA], indoleamine serotonin [5-HT] and 5-HT metabolite, 5-hydroxyindoleacetic acid [5-HIAA]) in discrete brain regions using high performance liquid chromatography (HPLC) equipped with an electrochemical detector. Female Wistar rats were exposed to 0, 50, 100, or 200 ppb gaseous GA or OPA by inhalation for 1 h per day, 5 d per week for 4 wk. Following the exposure, the brain of each rat was removed and dissected into cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum and hypothalamus. The neurotransmitters and their metabolites were extracted from each brain region, and determined by HPLC. Regarding GA, the daily water intake of the 50 or the 200 ppb exposed groups was significantly lower than that of the control. DA and 5-HIAA levels in the medulla oblongata among the GA exposed groups were significantly lower than those of the control. For OPA, the mean final body weight and daily food intake of the 100 or 200 ppb exposed groups were significantly lower than those of the control. The mean DA concentrations in the cerebrum in the groups exposed to OPA were significantly lower than those of the control. OPA may modulate DA metabolism in the cerebrum of female rats. The levels GA or OPA that induced alienations in neurotransmitters were comparable to those levels usually found in hospitals, further studies are warranted to evaluate the of safety of disinfectants containing GA or OPA.

Key words: Gluraraldehyde, *ortho*-phthalaldehyde, Dopamine, Neurotoxicity

Introduction

Glutaraldehyde (GA) has been used as a disinfectant for years[1)]. It has been the primary chemical used for a high degree of disinfection. Two per cent or 3% GA was most commonly used as a disinfectant for endoscope decontamination[1, 2)].

GA is a highly reactive chemical that has a specific smell. GA vapor stimulates mucous membranes in the eyes and respiratory tract. It is reported that the threshold for detecting smell is 0.04 ppm and that for stimulation of the mucous membrane is 0.3 ppm[3)]. Occupational hazards among hospital staffs exposed to

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GA in an endoscopy unit have been reported[4]. The symptoms observed among workers in endoscope disinfecting rooms were watery eyes, rhinitis, dermatitis, respiratory disorders, nausea and headache[5]. Occupational asthma has also been reported[6, 7]. The threshold limit value ceiling for GA is 0.05 ppm (0.2 mg/m^3) in the U.S.A[3]. In 2005, a guideline on management of disinfecting endoscopes in medical facilities was issued in Japan and the limited occupational exposure limit was set at 0.03 ppm in 2006.

The concern regarding the health effects of GA and the increasing frequency of GA-resistant *Mycobacterium chelonei* has recently led to development of a disinfectant containing *ortho*-phthalaldehyde (OPA). OPA has been demonstrated to be bactericidal and mycobactericidal and is now widely used for a high degree of disinfection for clinical apparatuses such as endoscopes as a substitute for GA[2]. OPA has been considered to be less toxic than GA. Lately, anaphylaxis following cytoscopy caused by a disinfectant containing OPA has been reported in several patients[8]. Moreover, OPA has been evaluated as a moderate eye and mucous membrane irritant[9]. The risk for exposure to OPA should also be evaluated.

Generally, increased lipid solubility enhances the rates of entry of a chemical into the central nervous system[10]. Therefore, organic compounds are possible neurotoxicants. Headache was reported as one of the symptoms of occupational GA exposure. Therefore, evaluation of the neurotoxicities of these compounds may be useful to evaluate the safety of GA and OPA.

The alterations in neurotransmitters and their metabolites catecholamines and indoleamines in brain regions have been used as indexes of neurotoxicities of toxicants[11–16]. The ratios of the levels of neurotransmitters to those of their metabolites have been used as indexes of neurotransmitter turnover and neuronal activity[17].

The objectives of the current study were to evaluate the neurotoxic effects of GA and OPA by inhalation. Rats were exposed to GA or OPA for 4 wk by inhalation as a model of actual exposure. The levels of neurotransmitters and their metabolites in discrete brain regions of the rats were then determined.

Materials and Methods

Animals

Female Wistar rats (6 wk of age) were purchased from Oriental Bioservice (Tokyo, Japan). The mean initial body weight of rats for GA exposure was 172.8 ± 2.3 g, (mean ± standard error) and that for OPA exposure was 155.0 ± 2.0 g. Rats were acclimated for 1 wk in a housing facility maintained at 24°C and humidity at 50% with a 10:14-h light/dark cycle before treatment. Rats were randomly assigned to the control groups and the treatment groups (5 rats per group), and housed in polycarbonate shoe-box style cages. There were no significant differences in the initial body weights among the groups exposed to GA or OPA. The food and water consumption of each group and body weight of each rat were monitored daily. All experimental procedures were performed in accordance with the Guidelines of Animal Care at Kitasato University.

Exposure to GA or OPA

Rats were exposed to 0, 50, 100 or 200 ppb of GA or OPA vapor in a self-made exposure chamber of a self-made exposure apparatus for 1 h per day, 5 d per week for 4 wk. The rats were exposed to GA or OPA in the mid morning. The exposure apparatus consisted of two steady-flow pumps (Minipump, Sibata, Tokyo, Japan), a water bath, a midget impinger, a filtration bottle, an exposure chamber, and processed PET bottles (Fig. 1). Rats were placed into the processed PET bottles, the mouths of which were cut for the noses of the rats, so that they were exposed to the air in the exposure chamber. A group (n=5 rats per group) was exposed to the respective concentration of each vapor simultaneously.

GA vapor was generated by bubbling 25% GA solution (Wako Pure Chemical Industries, Ltd, Osaka, Japan) incubated at 25°C in a water bath. A steady flow pump was used to cause the bubbling. The air for dilution was sent by another steady flow pump. The concentration of GA in the exposure chamber was adjusted by changing the flow volumes of the two pumps. OPA vapor was also generated by bubbling 0.55% OPA disinfectant (SC Johnson & Son, Racine, WI, U.S.A.).

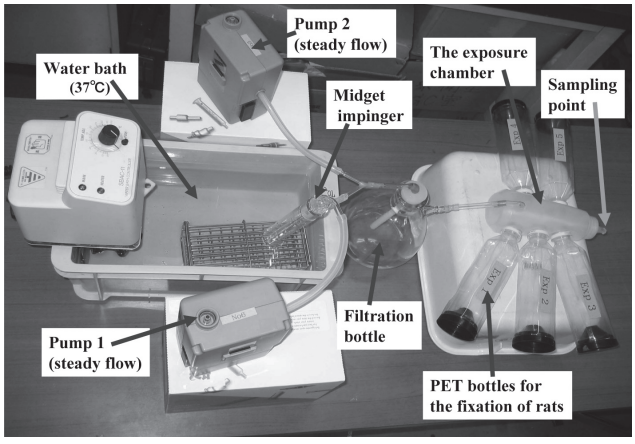

Fig. 1. Apparatus to detect exposure to GA or OPA.

Table 1. Range of concentrations of glutaraldehyde (GA) or *ortho*-phthalaldehyde (OPA) in the exposure chamber

Gases Set		Actual determination (ppb)	
	Concentration	Min	Max
GA	50 ppb	51.68	58.66
	100 ppb	102.74	115.32
	200 ppb	183.36	196.25
OPA	50 ppb	50.71	56.50
	100 ppb	91.56	102.30
	200 ppb	189.71	195.72

Determination of the concentrations of GA and OPA

The concentrations of GA or OPA in the exposure chambers were determined at the start and 5 min before the end of exposure (Table 1). The sampling point is shown in Fig. 1. The concentration of GA vapor in the exposure chamber was determined by high performance liquid chromatography (HPLC) (GL Sciences, Tokyo, Japan) after sampling using Sep-Pak dinitrophenylhydrazine (DNPH)-silica cartridges (Waters, Milford, MA, USA). Each sample was sampled for 5 min at the flow rate of 0.5 l/min. After sampling, GA was eluted by 2 ml of acetonitrile (Wako, Pure Chemical Industries). The eluted GA was determined by HPLC with a UV detector (wave length, 365 nm). A reverse-phase column (Inertsil C8-3, 4.6 × 250 mm, particle size 5 μm: GL Sciences) was used for chromatography. The mobile phase was composed of acetonitrile and water at the ratio of 7 to 3. Samples were eluted at 40°C at a flow rate of 0.7 ml/min. A calibration standard was obtained by using diluting solutions of GA-DNPH standard reagent (GL Sciences). The concentration of each sample was determined by the peak area method.

OPA was sampled using glass tube DNPH cartridges (SKC Inc., Eighty Four, PA, USA) for 5 min at the flow rate of 0.5 l/min. After sampling, DNPH was soaked in 2 ml of acetonitrile at 4°C overnight for desorption of OPA. The desorbed OPA was determined by HPLC with a UV detector, the reversed phase column and the mobile phase was done in the same manor for GA. The flow rate for the elution was 0.8 ml/min. For standard solution, 1 ml of 1 μg/ml of OPA solution and 0.8 mg/ml DNPH in acetonitrile were mixed and reacted at 4°C overnight. The standard curve for OPA was obtained by using diluted standard solution determined by the peak area method.

Determination of neurotransmitters

In the afternoon on the last day of the exposure period, each rat was decapitated, and brain and liver were removed and weighed. Each brain was dissected into six different regions (cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum, and hypothalamus) according to the method described by Glowinski and Iverson (1960). Brain samples were immediately soaked in ice-cold 0.05 M perchloric acid with 0.1% cysteine in tarred vials. After weighing, each sample was homogenized and centrifuged using 0.2 μm pore-size filter. The filtrate (sample) was stored at –80°C until analysis.

The concentrations of catecholamines (norepinephrine [NE], dopamine [DA], DA metabolites, dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]), indoleamine serotonin (5-hydroxytryptamine [5-HT]) and 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in each sample extract were determined simultaneously by HPLC with electrochemical detection (ECD) described previously[18]. The analytic system consisted of an electrochemical detector ED703 with a carbon electrode (GL Sciences), Hitachi model L-6300 pump (Hitachi, Tokyo), Gastorr model 722 degasser (Lab System, Ome), and Hitachi column oven 655A-52 with a temperature controller. A reversed phased column, Inertsil ODS-3, 4.6 × 150 mm, particle size 5 μm (GL Sciences) was employed for chromatography. The mobile phase composed of 9.6 g/l citric acid, 100 mg/l sodium octane sulfate, 40 mg/l EDTA and 15% of methanol. Samples were eluted at 35°C for 30 min at a flow of 1.0 ml/min.

A calibration standard containing NE bitartrate, DA hydrochloric acid, DOPAC, HVA, 5-HT creatinine sulfate and 5-HIAA dicyclohexylammonium salt (Sigma-Aldrich, St. Louis, MO, USA) in 0.05 M perchloric acid with 0.1% cysteine was employed. The detection limits were 5 ng/ml for 5-HT, 2.5 ng/ml for NE, DOPAC, HIAA and HVA, and 1.25 ng/ml for DA.

Statistical analyses

Ratios of DOPAC/DA, HVA/DA and 5-HIAA/5-HT were computed. Data were analyzed using one-way ANOVA (analysis of variance) followed by Fisher's PLSD (protected least significant difference) test using Statview J-5.0 software (SAS Institute, Cary, NC, USA). The mean values ± standard errors for each index in the group were indicated. $p<0.05$ indicated statistical significance.

Results

Daily intake of food or water of rats

There were no evident clinical signs observed over the period of exposure to GA. The mean daily intake of food or water of rats exposed to GA is shown in Fig. 2. There were no significant differences in the

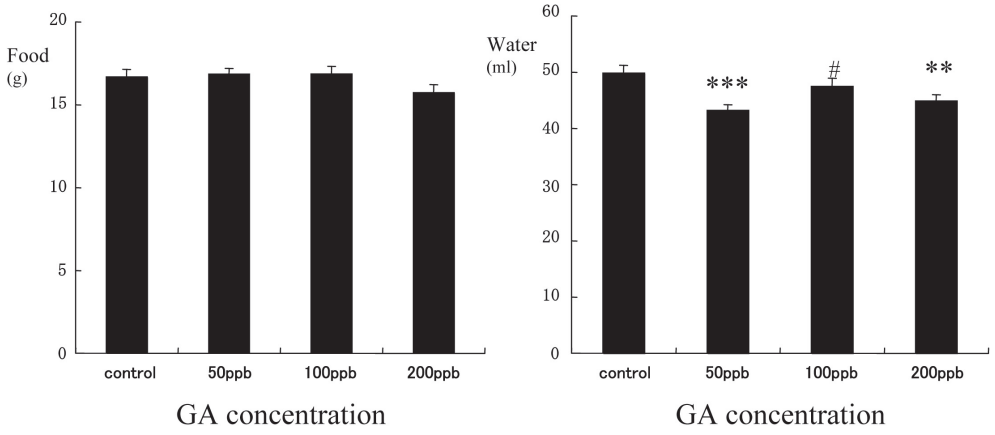

Fig. 2. Daily food or water intake of rats exposed to GA by inhalation for 4 wk. means ± SEs are indicated.

** and ***: $p<0.01$ and $p<0.001$ compared to the control, respectively.

#: $p<0.05$ compared to the 50 ppb group by Fisher's PLSD test.

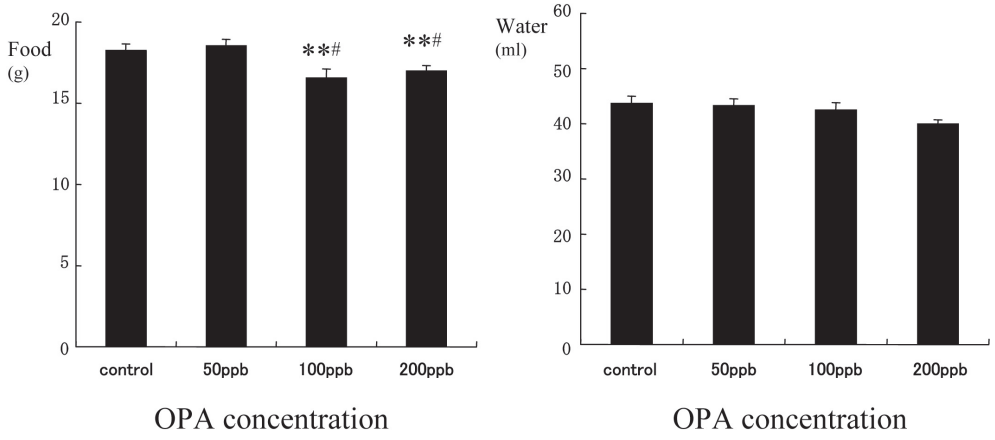

Fig. 3. Daily food or water intake of rats exposed to OPA by inhalation for 4 wk. means ± SEs are indicated.

**: $p<0.01$, compared to the control.

#: $p<0.05$ compared to the 50 ppb group by Fisher's PLSD test.

mean food consumption among the groups. The mean daily water intake of the 50 or 200 ppb exposed groups was significantly lower than that of the control. That of the 50 ppb exposed group was also significantly lower than that of the 100 ppb exposed group.

There were also no clinical signs observed over the period of exposure to OPA. The mean daily intake of food or water of rats exposed to OPA was demonstrated in Fig. 3. The mean daily food consumption of rats exposed to the 100 or 200 ppb exposed groups was significantly lower than that of the control or the 50 ppb exposed group. There were no significant differences in the mean water intake among the groups.

Body weight and relative liver weight

The mean body weight and relative liver weight of each exposed group of GA or OPA after the exposure period are shown in Fig. 4. There were no significant differences in the body weight or relative liver weight among the groups exposed to GA. While, the mean body weight of rats exposed to 100 or 200 ppb of OPA was significantly lower than that of the control. The mean relative liver weight of the 100 ppb exposed group was significantly higher than those of the control and other exposed groups.

Neurotransmitters and their metabolites

The concentrations of DA, 5-HT, or 5-HIAA in the medulla oblongata of the rats exposed to GA are shown in Fig. 5. The mean value of DA in the medulla oblongata of the rats in the 100 or 200 ppb groups was significantly lower than that of the control, and that of the

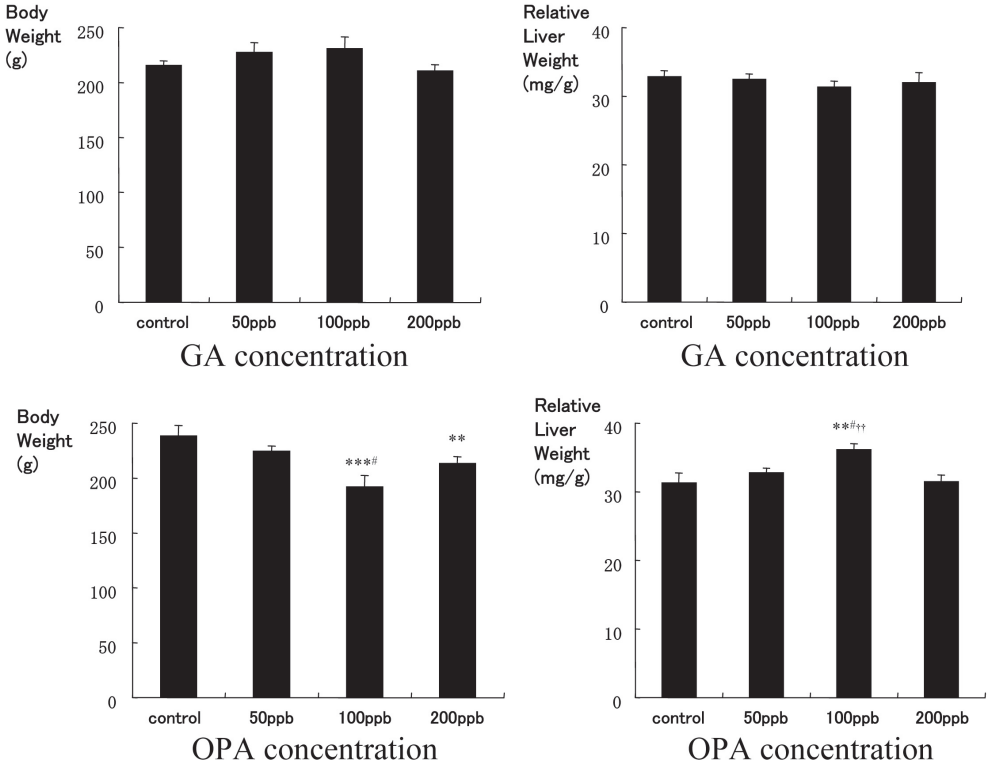

Fig. 4. Mean body weight and relative liver weight of rats exposed to GA or OPA by inhalation for 4 wk. means ± SEs are indicated.

** and ***: $p<0.01$ and $p<0.001$ compared to the control, respectively.

#: $p<0.05$ compared to the 50 ppb group, ††: $p<0.01$ compared to the 200 ppb group by Fisher's PLSD test.

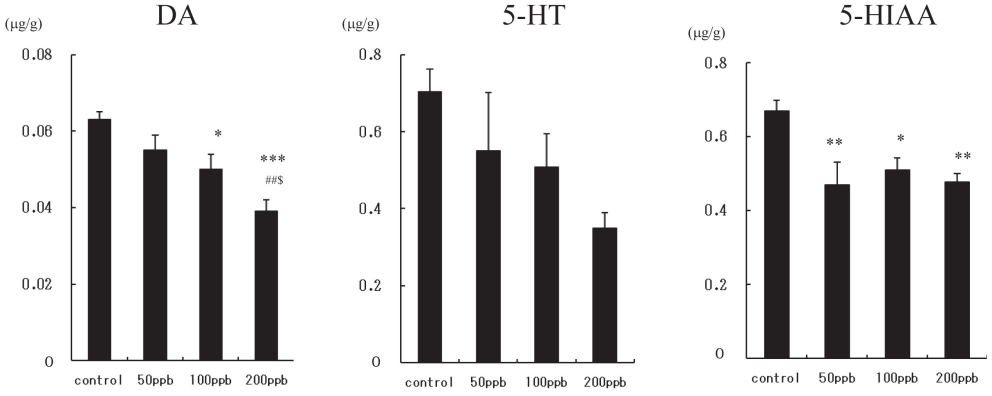

Fig. 5. Concentrations of DA, 5-HT, or 5-HIAA in the medullas of rats exposed to GA by inhalation for 4 wk. means ± SEs are indicated.

*, ** and ***: $p<0.05$, $p<0.01$ and $p<0.001$ compared to the control, respectively.

##: $p<0.01$ compared to the 50 ppb group, $: $p<0.05$ compared to the 100 ppb group by Fisher's PLSD test. $p=0.102$ by ANOVA for 5-HT.

200 ppb group was also significantly lower than that of the other exposed groups. Regarding 5-HT, although the mean values of the exposed groups were lower than that of the control, there were no significant differences among the groups. The mean values of 5-HIAA in the medulla oblongata of the rats in the groups exposed to GA were significantly lower than that of the control.

The concentrations of DA in various brain regions except for the medulla exposed to GA are given in Table 2. There were no significant differences in DA in these brain regions of the groups exposed to GA. There were no significant differences in DOPAC, HVA,

Table 2. Concentrations of DA in brain regions of rats exposed to glutaraldehyde (GA)

Group	Cerebrum	Cerebellum	Midbrain	Striatum	Hypothalamus
Control	1.077 ± 0.122	0.009 ± 0.001	0.304 ± 0.085	0.358 ± 0.100	1.688 ± 0.541
GA 50 ppm	0.852 ± 0.267	0.011 ± 0.002	0.323 ± 0.122	0.500 ± 0.137	1.749 ± 0.342
GA 100 ppm	1.160 ± 0.324	0.014 ± 0.004	0.294 ± 0.078	0.212 ± 0.020	2.367 ± 0.706
GA 200 ppm	1.189 ± 0.176	0.009 ± 0.001	0.356 ± 0.054	0.401 ± 0.069	1.041 ± 0.141

Note: Means (μg/g) ± SEs are indicated.

Table 3. Concentrations of DOPAC, HVA and NE in various brain regions of rats exposed to GA

Neuro-chemical	Group	Cerebrum	Cerebellum	Medulla	Midbrain	Striatum	Hypothalamus
DOPAC	Control	0.616 ± 0.278	0.073 ± 0.008	0.057 ± 0.003	0.277 ± 0.076	0.145 ± 0.035	0.462 ± 0.100
	GA 50 ppm	0.307 ± 0.100	0.067 ± 0.009	0.050 ± 0.006	0.265 ± 0.075	0.164 ± 0.051	0.449 ± 0.085
	GA 100 ppm	0.311 ± 0.078	0.051 ± 0.012	0.051 ± 0.002	0.188 ± 0.024	0.078 ± 0.008	0.424 ± 0.073
	GA 200 ppm	0.236 ± 0.090	0.069 ± 0.000	0.047 ± 0.062	0.339 ± 0.062	0.136 ± 0.025	0.506 ± 0.188
HVA	Control	0.318 ± 0.126	0.009 ± 0.001	0.043 ± 0.002	0.101 ± 0.024	0.058 ± 0.014	0.301 ± 0.111
	GA 50 ppm	0.193 ± 0.063	0.011 ± 0.002	0.045 ± 0.004	0.078 ± 0.021	0.069 ± 0.017	0.367 ± 0.110
	GA 100 ppm	0.154 ± 0.036	0.014 ± 0.004	0.042 ± 0.006	0.055 ± 0.006	0.047 ± 0.013	0.412 ± 0.155
	GA 200 ppm	0.139 ± 0.039	0.009 ± 0.001	0.031 ± 0.003	0.094 ± 0.016	0.061 ± 0.014	0.202 ± 0.049
NE	Control	0.558 ± 0.219	0.223 ± 0.017	0.798 ± 0.025	0.507 ± 0.135	0.650 ± 0.078	7.169 ± 1.452
	GA 50 ppm	0.582 ± 0.170	0.223 ± 0.020	0.770 ± 0.061	0.494 ± 0.055	0.616 ± 0.055	8.716 ± 0.586
	GA 100 ppm	0.324 ± 0.021	0.205 ± 0.033	0.753 ± 0.053	0.669 ± 0.149	0.618 ± 0.039	8.233 ± 1.012
	GA 200 ppm	0.365 ± 0.029	0.173 ± 0.037	0.621 ± 0.043	0.658 ± 0.126	0.504 ± 0.035	7.586 ± 1.013

Note: Means (μg/g) ± SEs are indicated.

Table 4. Concentrations of 5-HT and 5-HIAA in various brain regions of rats exposed to GA

Neuro-chemical	Group	Cerebrum	Cerebellum	Midbrain	Striatum	Hypothalamus
5-HT	Control	0.526 ± 0.309	0.049 ± 0.011	0.458 ± 0.141	0.538 ± 0.048	1.769 ± 0.399
	GA 50 ppm	0.325 ± 0.053	0.069 ± 0.006	0.423 ± 0.127	0.604 ± 0.037	1.862 ± 0.398
	GA 100 ppm	0.250 ± 0.017	0.059 ± 0.017	0.679 ± 0.280	0.571 ± 0.025	1.348 ± 0.406
	GA 200 ppm	0.337 ± 0.044	0.058 ± 0.003	0.712 ± 0.301	0.532 ± 0.039	2.383 ± 0.373
5-HIAA	Control	1.148 ± 0.469	0.430 ± 0.059	0.705 ± 0.129	0.469 ± 0.070	1.036 ± 0.129
	GA 50 ppm	0.608 ± 0.240	0.423 ± 0.046	0.590 ± 0.076	0.447 ± 0.044	0.983 ± 0.170
	GA 100 ppm	0.353 ± 0.029	0.357 ± 0.087	0.633 ± 0.120	0.373 ± 0.032	0.597 ± 0.172
	GA 200 ppm	0.334 ± 0.031	0.390 ± 0.090	0.682 ± 0.116	0.327 ± 0.042	1.188 ± 0.351

Note: Means (μg/g) ± SEs are indicated.

NE (Table 3), 5-HT, or 5-HIAA levels (Table 4) in any brain regions among the groups exposed to GA.

The concentrations of DA and DA metabolites in the cerebrum of rats exposed to OPA were demonstrated in Fig. 6. The mean value of DOPAC in the cerebrum of the 100 or 200 ppb was significantly lower than that of the control. The mean value of HVA in the cerebrum of the 100 or 200 ppb was also significantly lower than that of the control. There were no significant differences in DA in the cerebrum among the groups. The concentrations of DA, DOPAC, and HVA in various brain regions except for the cerebrum of rats exposed to OPA are given in Table 5. There were no significant differences among the groups. There were no significant differences in NE (Table 6), 5-HT, or 5-HIAA levels (Table 7) in any region among the groups exposed to OPA.

And there were no significant differences in DOPAC/DA, HVA/DA, or 5-HIAA/5-HT in any of the brain regions among the rats exposed to GA or OPA.

Discussion

In Japan, the number of endoscopies performed in

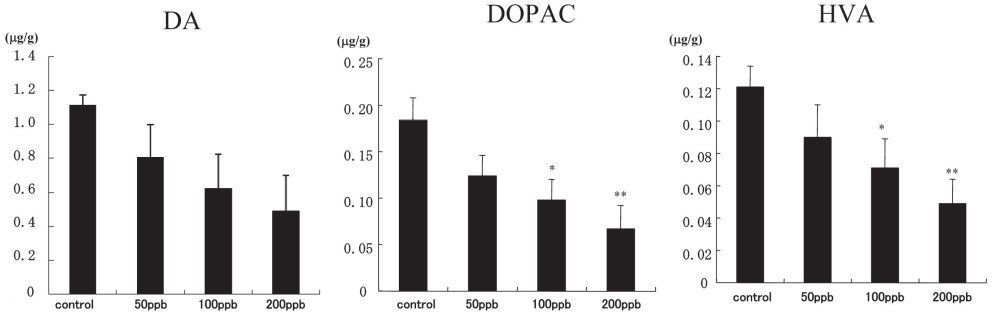

Fig. 6. Concentrations of DA or DA metabolites (DOPAC, HVA) in the cerebrums of rats exposed to OPA by inhalation for 4 wk.
means ± SEs are indicated.
* and **: $p<0.05$ and $p<0.01$ compared to the control by Fisher's PLSD test, respectively. $p=0.108$ by ANOVA for DA.

Table 5. Concentrations of DA, DOPAC and HVA in various brain regions of rats exposed to OPA

Neuro-chemicals	Group	Cerebellum	Medulla	Midbrain	Striatum	Hypothalamus
DA	Control	0.006 ± 0.001	0.058 ± 0.006	0.418 ± 0.108	0.679 ± 0.135	0.713 ± 0.103
	OPA 50 ppm	0.006 ± 0.001	0.049 ± 0.004	0.556 ± 0.167	0.481 ± 0.113	1.052 ± 0.324
	OPA 100 ppm	0.007 ± 0.002	0.051 ± 0.003	0.510 ± 0.159	0.476 ± 0.138	1.091 ± 0.394
	OPA 200 ppm	0.005 ± 0.001	0.051 ± 0.005	0.614 ± 0.113	0.579 ± 0.165	1.047 ± 0.403
DOPAC	Control	0.020 ± 0.006	0.088 ± 0.017	0.107 ± 0.013	0.176 ± 0.034	0.187 ± 0.054
	OPA 50 ppm	0.018 ± 0.006	0.113 ± 0.045	0.181 ± 0.050	0.112 ± 0.023	0.200 ± 0.045
	OPA 100 ppm	0.020 ± 0.006	0.093 ± 0.022	0.167 ± 0.050	0.129 ± 0.032	0.208 ± 0.065
	OPA 200 ppm	0.017 ± 0.005	0.103 ± 0.037	0.194 ± 0.045	0.172 ± 0.051	0.202 ± 0.069
HVA	Control	0.016 ± 0.002	0.037 ± 0.004	0.056 ± 0.007	0.081 ± 0.014	0.143 ± 0.021
	OPA 50 ppm	0.021 ± 0.002	0.036 ± 0.004	0.079 ± 0.021	0.063 ± 0.008	0.160 ± 0.012
	OPA 100 ppm	0.018 ± 0.004	0.039 ± 0.005	0.058 ± 0.011	0.063 ± 0.011	0.183 ± 0.021
	OPA 200 ppm	0.017 ± 0.004	0.056 ± 0.016	0.088 ± 0.022	0.072 ± 0.016	0.132 ± 0.016

Note: Means (μg/g) ± SEs are indicated.

Table 6. Concentrations of NE in various brain regions of rats exposed to OPA

Group	Cerebrum	Cerebellum	Medulla	Midbrain	Striatum	Hypothalamus
Control	0.298 ± 0.010	0.282 ± 0.017	0.635 ± 0.046	0.480 ± 0.056	0.485 ± 0.096	5.047 ± 1.632
GA 50 ppm	0.295 ± 0.022	0.288 ± 0.025	0.589 ± 0.025	0.475 ± 0.034	0.572 ± 0.095	4.924 ± 1.509
GA 100 ppm	0.334 ± 0.027	0.331 ± 0.038	0.676 ± 0.061	0.480 ± 0.043	0.561 ± 0.139	5.200 ± 1.701
GA 200 ppm	0.295 ± 0.067	0.286 ± 0.033	0.629 ± 0.047	0.501 ± 0.054	0.479 ± 0.079	4.945 ± 1.435

Note: Means (μg/g) ± SEs are indicated.

Table 7. Concentrations of 5-HT and 5-HIAA in various brain regions of rats exposed to OPA

Neuro-chemical	Group	Cerebrum	Cerebellum	Medulla	Midbrain	Striatum	Hypothalamus
5-HT	Control	0.443 ± 0.101	0.077 ± 0.009	0.669 ± 0.055	0.570 ± 0.128	0.576 ± 0.056	1.575 ± 0.427
	GA 50 ppm	0.336 ± 0.045	0.071 ± 0.010	0.569 ± 0.037	0.535 ± 0.090	0.567 ± 0.033	1.614 ± 0.396
	GA 100 ppm	0.352 ± 0.124	0.071 ± 0.007	0.658 ± 0.054	0.627 ± 0.068	0.512 ± 0.035	1.613 ± 0.376
	GA 200 ppm	0.326 ± 0.093	0.071 ± 0.010	0.593 ± 0.049	0.517 ± 0.093	0.472 ± 0.030	1.709 ± 0.391
5-HIAA	Control	0.260 ± 0.032	0.241 ± 0.095	0.410 ± 0.073	0.589 ± 0.038	0.352 ± 0.064	0.977 ± 0.482
	GA 50 ppm	0.271 ± 0.030	0.300 ± 0.130	0.445 ± 0.092	0.612 ± 0.052	0.378 ± 0.058	0.773 ± 0.338
	GA 100 ppm	0.252 ± 0.016	0.308 ± 0.135	0.409 ± 0.060	0.584 ± 0.074	0.334 ± 0.035	0.761 ± 0.345
	GA 200 ppm	0.269 ± 0.077	0.266 ± 0.119	0.410 ± 0.091	0.589 ± 0.050	0.380 ± 0.059	0.822 ± 0.366

Note: Means (μg/g) ± SEs are indicated.

hospitals was far more compared to that in other countries because of the high prevalence of disease in digestive organs such as gastric cancer[19]. It is also likely due to the fact that everyone in Japan is covered by the national health insurance making endoscopic procedures much less expensive than they are in most other countries. Therefore, quick washing and disinfection of the endoscopic apparatuses are required. GA or OPA was used for a high degree of disinfection within a short time period. Symptoms induced by the inhalation of GA have been reported[4, 6, 7]. Regarding OPA, which has been sold in retail markets as a substitute for GA since 2002, its stimulation to the nose, throat, and respiratory tract has also been reported[8].

In addition to the stimulation by GA or OPA, headache has been reported as one of the symptoms induced by the exposure to GA. Jachuck *et al*. reported that workers in endoscopy rooms show symptoms such as stimulation to the nose and eyes, dermatitis, dyspnea, nausea, and headache caused by the exposure to 50 to 120 ppb of GA for 1 h or over 200 ppb of GA for a short term[4]. It is of interest whether headache caused by the exposure to GA is a specific or general symptom. As a substitute for GA, the neurotoxic effects of OPA should also be examined because of its chemical characteristic as an organic compound. The workers in endoscopy rooms who show clinical symptoms were mainly females for GA[20] and OPA[21]. Therefore, we examined the neurotoxicity of GA and OPA using a model of female rats that had inhaled GA or OPA.

The concentrations of GA or OPA in the exposure chamber were stably maintained by our generator using HPLC for determination. The actual values of GA and OPA were close to the concentrations to be set.

We monitored body weight and intake of food or water daily to examine the effects on appetite and the development of the rats by the inhalation of GA or OPA. Regarding GA, the mean value of daily water intake of the groups exposed to 50 or 200 ppb GA was significantly lower than that of the control, although it was not a dose-dependent effect. The cause for the decrease in the rats' water intake was not clear. However, there were no alterations in daily food intake among the groups exposed to GA or the control.

For the effects of GA on development, there were no alterations in body weight among the rats exposed GA or the control. In a previous study, van Birgelen *et al*. exposed F344/N rats to 250, 500, 750 ppb of GA vapor by inhalation for 6 h per day, 5 d a week for a total of 104 wk[22]. The mean body weight of the 500 or 750 ppb exposed group was significantly lower than that of the control. Zissu *et al*. exposed 100 ppb of GA vapor to male and female B6C3F1 mice aged 6 wk by inhalation for 6 h per day, 5 d a week for total 52 or 78 wk[23]. For the female mice, the mean values of body weights in both the groups exposed to GA for 52 and 78 wk were significantly lower than those of the respective controls. However, the mean values of the body weights of the male mice exposed to GA were significantly lower than those of the respective control. The effects of high-dose GA on body weight gain were not the same as those in previous studies. The low-dose exposure to GA for 4 wk, i.e., shorter than that in previous studies, in the present study did not affect body weight.

Regarding neurotransmitters and their metabolites in discrete brain regions of rats exposed to GA, the only significant differences observed were in medulla oblongata for DA and 5-HIAA. In medulla oblongata, the concentrations of DA were quite low, and there were no major DA pathways[24]. However, in addition to the significantly lower mean values of 5-HIAA in the GA-exposed groups, the mean values of 5-HT in the medulla oblongata were lower than that of the control. As there were no significant differences in the 5-HIAA/5-HT ratio in the medulla oblongata, the 5-HT synthesis may be inhibited by GA in the medulla. However, the reason the differences were limited in the medulla oblongata was not clear.

Regarding OPA, the mean body weight of rats exposed to the 100 or 200 ppb groups was significantly lower than that of the control. OPA may induce appetite loss and inhibit rat development. Daily food intake of the 100 and 200 ppb exposed groups was significantly lower than that of the control. For the relative liver weight, the mean value of the 100 ppb exposed group was significantly higher than that of the control. This may be due to the decrease in body weight. Further studies are warranted to determine the effects of OPA by inhalation on the development of rats.

The DA systems are well-known modulators of activity and behavior[24] and related to food intake[25] and water intake[26]. In the present study, the mean values of DA in the cerebrum in the groups exposed to OPA were significantly lower than that in the control. Since the alterations in the ratios of DA metabolites to DA did not reach the significant level, the DA synthesis may be decreased in the cerebrum by the inhalation of OPA. Also, the decrease in DA in the cerebrum may be due to the network via the olfactory nerve. This decrease in DA in the cerebrum may be related to the significantly lower intake of food. However, there were no significant differences in DA or DA metabolite levels in any other brain regions.

OPA has been considered to be less toxic than GA. Although it does not commonly occur in various brain

regions, decreases in DA metabolites were observed in the cerebrum of OPA exposed rats. OPA may be neurotoxic at the concentrations used with inhalation in the present study. It is of interest whether the decreases in DA metabolism observed here are related to the alterations in behavior or not. Future behavioral tests may be warranted.

The levels used in this study are comparable to the levels in hospitals. Because OPA is widely used as a disinfectant for endoscopic apparatuses, it is necessary to set the threshold limit value-ceiling for OPA for the safety of the workers. At present, keeping low levels avoiding the exposure of OPA may be a good countermeasure for any possible ill health effects of OPA. To avoid exposure, protective devices are always useful. Training workers is also important to avoid unnecessary exposure to disinfectants.

In the present study, OPA may effect DA metabolism in the cerebrum of rats in addition to their body weight. The effects of GA were observed in the medulla, and the groups exposed to GA showed lower daily water intake. The neurotoxicity of disinfectants warrants further studies.

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