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Original Article

Effects of Perinatal Exposure to Bisphenol A on Brain Neurotransmitters in Female Rat Offspring

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Abstract: Pregnant Sprague-Dawley (CD IGS) rats were orally administered doses of bisphenol A (BPA) at 4, 40, and 400 mg/kg, from gestation days 6 to postnatal day 20. Neurotransmitters such as dopamine (DA) and serotonin (5HT) were extracted from the brains of dams and female offspring, and measured using liquid chromatography. BPA at 400 mg/kg was toxic and dosed rats died. At 3 wk after birth, brain levels of 3,4-dihydroxyphenylacetic acid (DOPAC, a DA metabolite), homovanillic acid (HVA, a DA metabolite), 5HT, 5-hydroxyindoleacetic acid (5HIAA, a 5HT metabolite) in female offspring were increased and the HVA/DA ratio was high in some brain areas of BPA-treated groups as compared with controls. At the age of 6 wk, levels of choline (Ch) in BPA-treated groups at 4 and 40 mg/kg were higher than control in all of eight brain areas. No changes were observed in acetylcholine (ACh) contents. In 9-wk-old offspring, changes in monoamines and metabolites were scattered and not great. At 3 wk after delivery, levels of 5HIAA in some brain areas of dams treated with BPA were higher than in control dams. Dose dependent increases in HVA and the HVA/DA ratio of the occipital cortex, and in the HVA/DA ratio of the frontal cortex were observed. The turnover of DA and 5HT was accelerated in 3-wk-old offspring and dams. BPA possesses very weak estrogenic activity. Changes in cerebral neurotransmitters observed in offspring and dams in this study may have been related to the estrogenic activity of BPA. However, further investigation is needed to examine the contribution of hormonal activity to such neurotransmitter changes.

Key words: Bisphenol A, Perinatal exposure, Offspring, Brain, Neurotransmitters, Dopamine, Serotonin, Acetylcholine, IGS rat

Introduction

Among many stabilizers of plastics, bisphenol A (BPA) is a popular stabilizer that mimics the actions of estrogen and affects the endocrine glands *in vivo* and *in vitro*[1, 2]. Although BPA binds to estrogen receptors to a lesser extent than 17β-estradiol, BPA affects sperm production and the prostate in male offspring, as well as body weight in male and female offspring[3–5]. Low dose effects of BPA and inverted U-shaped dose response relationships have also been reported at 2 to 20 μg/kg and at 0.1 to 50 mg/kg, respectively[6, 7]. The nervous systems of fetuses and newborns are susceptible to chemical effects[8, 9], and the maternal administration of BPA affects the reproductive system and behavior of experimental animal's offspring[10, 11]. These reports strongly suggest that maternal administration of BPA affects the nervous system of offspring. We previously examined how the maternal administration of BPA affects the reproductive organs, sex hormones, learning and memory functions of offspring[12, 13]. We found that the plasma testosterone concentrations of rats at 9 wk of age were significantly elevated in BPA groups compared with

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controls[13]. The content of testosterone in the testes increased in a similar manner to that in plasma. We also studied neurochemical changes in the neonatal brain. Neurotransmitters play key roles in the regulation of brain function. Many mental and nervous diseases are related to disordered function of neurotransmitters, and neuroactive drugs act by altering neurotransmitter levels[14]. Neurochemical changes are also involved in chemical neurotoxicity[15, 16].

The present study used a neurochemical approach to investigate how BPA alters brain function in second generation rats. Following the maternal administration of BPA during pregnancy and lactation, we assayed the brain for contents of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite), homovanillic acid (HVA, a dopamine metabolite), serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA, a 5HT metabolite), acetylcholine (ACh), and choline (Ch, an ACh precursor and metabolite) in female rat offspring.

Materials and Methods

Animals and chemicals

The CD (SD) IGS strain of rats was used, and 24 pregnant 9-wk-old rats were purchased from Charles River Japan Inc. at gestation day (GD) 3. GD 0 was confirmed by the presence of a copulatory plug. They were individually housed under a 12/12 h light/dark cycle with lights on at 08:00, with free access to feed (CE-2, Japan Clea, Inc.) and tap water. Room temperature and humidity were maintained at 23 ± 1°C and 55 ± 5%, respectively. Four rat groups, of 6 pregnant rats each, were given standard BPA (>99.8% pure; Cat#: 280-08561, Lot#: HCE9312, Wako Pure Chemicals, Japan), at 0 (control), 4, 40, or 400 mg/kg body weight (BW), respectively. BPA was dissolved in corn oil (10 ml/kg BW).

Administration of BPA to pregnant rats

BPA was administered to rats by oral gavage between 08:30 and 09:30 from GD 6 through postnatal day (PND) 20. The day of birth by 10:00 was considered PND 0. One dam in the control group was not pregnant. Therefore, 5 dams were available for the analysis in the control group. In the 4 and 40 mg/kg BPA groups, 6 dams were available for the analysis, but in the rat group administered with daily doses of BPA at 400 mg/kg, 4 rats died before and after delivery. Therefore, the rat group given 400 mg/kg BPA was not used in the analysis. Pups were sacrificed at 1, 3, 6, and 9 wk of age between 13:00 and 16:00. The litter size was standardized to 10 pups (male:female = 5:5, if possible) for each dam on PND 7. Subsequently, at 3, 6 and 9 wk of age, 4 to 6 pups of each sex were sacrificed from each BPA dose group. Pups chosen for sacrifice in each of the BPA dose groups were culled from different dams. Because of the imbalance of male and female numbers and different pup numbers among dams, some pups remained after the litter size standardization on PND 7. These pups were used for the analysis at 1 wk of age. Therefore, the numbers of male and female pups sacrificed in each dose group at 1 wk of age ranged from 1 to 10.

Offspring were weaned on PND 21, and males and females were separately housed. The highest dose, 400 mg/kg BPA was selected after Kwon *et al.*[17], who observed no effects of BPA at doses of 320 mg/kg/day from GD 11 through PND 20 on maternal body weight. The brain contents of neurotransmitters of offspring were assayed at 1, 3, 6, and 9 wk after birth. The brain neurotransmitters of dams given BPA were assayed at 3 wk after delivery (15 wk old).

Extraction and measurement of brain substances

Pups were sacrificed by decapitation under ice-cold hypothermia at 1 wk after birth to obtain organs in addition to the brain. At 3 and 9 wk after birth, pups were sacrificed by exsanguination from the abdominal vein under ether anesthesia to obtain organs including the brain. No effects of ether anesthesia on the brain monoamines were confirmed. At 6 wk after birth, the pups were sacrificed by microwave exposure (1.5 KW, 0.8 s) focused on the head (Microwave applicator, Muromachi Kikai Co., Tokyo)[18]. Exposure to microwaves rapidly increases the brain temperature and prevents rapid postmortem changes of brain substances. At 1, 3 and 9 wk after birth, microwaves were not used for sacrifice to obtain the other organs. Therefore, the ACh and Ch contents could not be measured in offspring at these ages. At 1 wk after birth, brain substances were analyzed in the whole brain because the brain was too small to divide exactly into individual regions. Three wk after birth, the brains (half brain) were dissected on ice into the forebrain, hindbrain, medulla oblongata, and cerebellum. The forebrain and hindbrain were obtained by cutting the brain vertically at the level of the optic chiasm after removing the cerebellum and medulla oblongata. At 6 wk after birth, the brains were dissected into the frontal cortex, occipital cortex, hippocampus, midbrain, striatum, hypothalamus, medulla oblongata, and cerebellum as described by Glowinski and Iversen[19]. Nine wk after birth, half the brain was used for the measurement of enzyme activity and the other half of the brain was dissected into the four brain regions like the

brain of 3-wk-old rats, to measure monoamine contents. Brains of dams were dissected into eight brain regions as described above according to Glowinski and Iversen. Dams were sacrificed on the day of the weaning of pups between 13:00 and 16:00. They were exsanguinated from the abdominal vein under ether anesthesia to obtain organs in addition to the brain. All brain samples were stored at –80°C and dissolved in 0.2 N $HClO_4$ containing 1 mM EDTA and 5 mM $Na_2S_2O_5$ before disruption using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 12,000 g for 25 min at 4°C, and the supernatant was analyzed by HPLC[20, 21]. Supernatants were divided into two portions (Portion A & B). Portion A was neutralized with potassium acetate and the supernatant was obtained after centrifugation. This supernatant was analyzed by HPLC to determine ACh and Ch contents. Portion B was applied to activated alumina to adsorb NE, DA, and DOPAC (Portion B1). HVA, 5HT, and 5HIAA remained in the eluate after centrifugation (Portion B2). Each portion was passed through a filter of 0.45 μm pore size before application to HPLC.

HPLC analysis

ACh and Ch in portion A and ethylhomocholine (internal standard), were separated by reverse phase ion pair chromatography (Eicompak AC-GEL, Eicom Co., Japan) using a mobile phase comprising 0.1 M phosphate buffer, pH 8.2[22]. One liter of this buffer contained sodium 1-decanesulfonate, tetramethylammonium chloride, and EDTA·Na_2·H_2O. Eluates were passed through a column that fixed ACh esterase and Ch oxidase (AC Enzymepak, Eicom). The column temperature was maintained at 30°C in an oven. The flow rate of the HPLC pump (L-4000, Hitachi Co., Tokyo) was 0.6 ml/min. ACh, Ch, and ethylhomocholine were assayed using an electrochemical detector (ECD-100, Eicom) equipped with a platinum electrode to measure the amount of H_2O_2 produced by the enzyme reaction of the three compounds. The voltage for electrochemical detection was 450 mV.

Monoamines and metabolites were assayed in extracts from the brain homogenates by HPLC equipped with an electrochemical detector (ECD-300, Eicom) and a carbon electrode. Reverse phase ion pair chromatography separated NE, DA, and DOPAC in portion B1 (Eicompak MA-5ODS, Eicom). The mobile phase was citrate-acetate buffer, pH 3.5. The retention time for each component was adjusted by adding sodium 1-octanesulfonate and methanol. An Eicompak MA-5ODS separated HVA, 5HT, and 5HIAA in portion B2 using a mobile phase comprising citrate-acetate buffer, pH 3.9. The separation parameters were as follows: temperature, 25°C; flow rate, between 0.5 and 0.9 ml/min; voltage, between 700 and 800 mV.

Statistics

Means ± SEM of each group were calculated for each of the monoamine or metabolite contents of the brain (nmoles/g tissue). Amine ratios (DOPAC/DA, HVA/DA, 5HIAA/5HT, and ACh/Ch) were calculated for each rat and the mean values of these ratios were obtained for each group. Metabolite/monoamine ratios (DOPAC/DA, HVA/DA, and 5HIAA/5HT) are widely used as markers of turnover of DA and 5HT in cerebral neurons. The statistical significance of differences between the control and dosed groups was examined by Dunnett's multiple *t*-test using statistics software (SPSS Japan Inc.). Differences between groups at $p<0.05$ were considered significant.

Results

Effects of BPA on 1-wk-old offspring

Figure 1 shows how the maternal administration of BPA affected the brain content of neurotransmitters and metabolites in female offspring at 1 wk after birth. In BPA-treated groups, levels of DOPAC and 5HT were low and those of DA and HVA were high, although these changes were less than 20% of each control value and no differences were statistically significant. Metabolite/monoamine ratios (DOPAC/DA, HVA/DA, and 5HIAA/5HT) were calculated for each rat. A significant difference was observed between the DOPAC/DA ratios of the control (0.264, 100%) and 40 mg/kg (0.200, 75.8%) groups. No significant differences were found between the control and BPA-treated groups in other metabolite/monoamine ratios.

Effects of BPA on the offspring at 3 wk of age

Figures 2-1 to 2-3 show levels of neurotransmitters, metabolites, and ratios of DOPAC/DA, HVA/DA, and 5HIAA/5HT in the brains of 3-wk-old rats. Levels of neurotransmitters in the cerebellum were low and the data varied too much to perform statistical analyses. Therefore, data obtained for the cerebellum are not presented. No effects of BPA were observed on the contents of NE and DA in the forebrain, hindbrain, and medulla oblongata. Levels of DOPAC of the 40 mg/kg group and HVA of the 4 mg/kg group were significantly increased in the forebrain compared with controls. A statistical significance was found in the difference of the mean values of the HVA/DA ratio in the forebrain between the control (0.161, 100%) and 4 mg/kg (0.280, 174%) groups. Levels of 5HT and 5HIAA in the

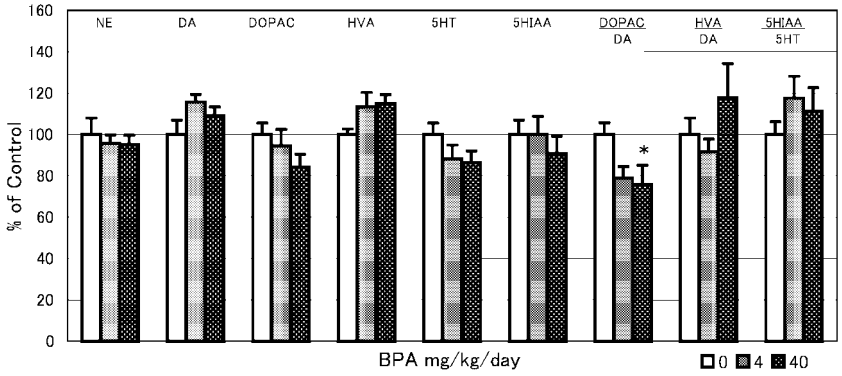

Fig. 1. Effects of perinatal administration of BPA on the neurotransmitter contents of whole brain in 1-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.736 for NE, 1.18 for DA, 0.305 for DOPAC, 0.575 for HVA, 11.8 for 5HT, and 2.69 for 5HIAA; absolute values of ratios for 100% were as follows: 0.264 for DOPAC/DA, 0.522 for HVA/DA, and 0.228 for 5HIAA/5HT. N = 6–8. *: $p<0.05$ by Dunnett's multiple *t*-test.

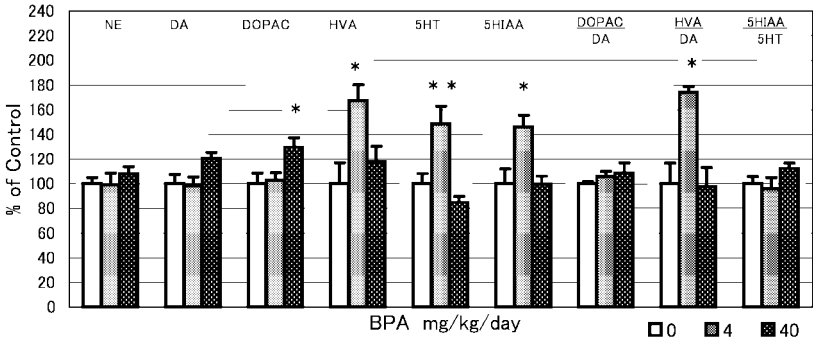

Fig. 2-1. Effects of perinatal administration of BPA on the neurotransmitter contents of forebrain in 3-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.944 for NE, 6.59 for DA, 1.30 for DOPAC, 1.08 for HVA, 1.64 for 5HT, and 1.12 for 5HIAA; absolute values of ratios for 100% were as follows: 0.197 for DOPAC/DA, 0.161 for HVA/DA, and 0.717 for 5HIAA/5HT. N = 4–5. *: $p<0.05$; **: $p<0.01$ by Dunnett's multiple *t*-test.

forebrain of the 4 mg/kg group were significantly increased compared with controls. There was no difference in the 5HIAA/5HT ratio in the forebrain between the control and 4 mg/kg groups. The level of HVA in the hindbrain of the 40mg/kg group was higher than that of the control (130% of the control). The HVA/DA ratios in the hindbrain of BPA-treated groups were higher than that of the control (120 and 132% of the control at 4 and 40 mg/kg, respectively), although statistical significance was not found in these differences. The decrease in 5HT in the hindbrain of BPA-treated groups was within 20% of the control, and was statistically significant for the 40 mg/kg group. There were no significant changes in the 5HIAA/5HT ratios in the hindbrains of the 4 and 40 mg/kg groups. In the medulla oblongata, levels of HVA of the 40 mg/kg group, and 5HT and 5HIAA of the 4 and 40 mg/kg groups, were higher than those of the control. Among them 5HT of the 4 mg/kg group was significantly increased compared with the control. The HVA/DA ratio of the 40 mg/kg group was higher than that of the control, however, the difference was not statistically significant.

Effects of BPA on the offspring at 6 wk of age

Tables 1-1 to 3-2 summarize the results of neurotransmitter analysis of offspring at 6 wk after birth.

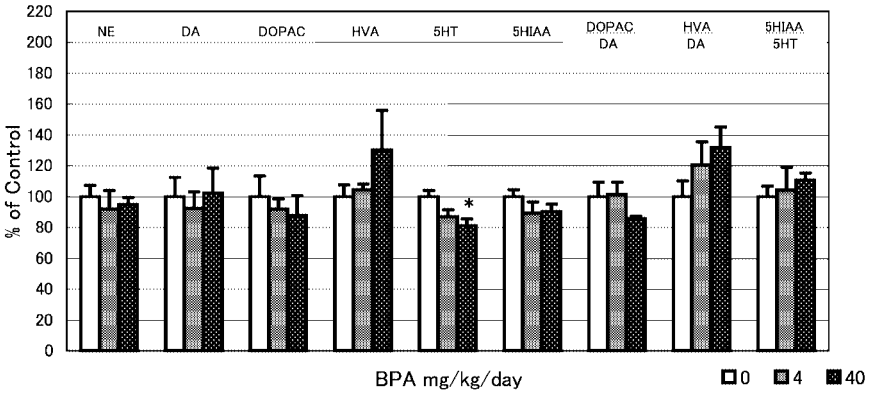

Fig. 2-2. Effects of perinatal administration of BPA on the neurotransmitter contents of hindbrain in 3-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.32 for NE, 1.08 for DA, 0.272 for DOPAC, 0.206 for HVA, 11.9 for 5HT, and 3.32 for 5HIAA; absolute values of ratios for 100% were as follows: 0.254 for DOPAC/DA, 0.188 for HVA/DA, and 0.281 for 5HIAA/5HT. N = 4–5. *: $p<0.05$ by Dunnett's multiple *t*-test.

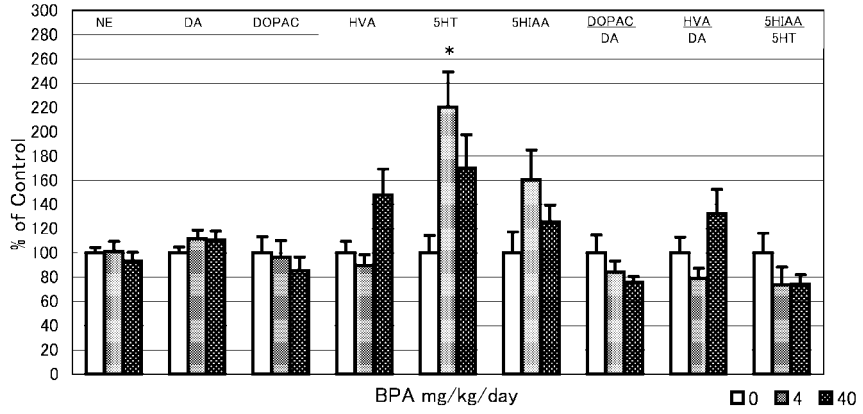

Fig. 2-3. Effects of perinatal administration of BPA on the neurotransmitter contents of medulla oblongata in 3-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 2.56 for NE, 0.193 for DA, 0.106 for DOPAC, 0.214 for HVA, 3.14 for 5HT, and 3.63 for 5HIAA; absolute values of ratios for 100% were as follows: 0.556 for DOPAC/DA, 1.14 for HVA/DA, and 1.20 for 5HIAA/5HT. N = 4–5. *: $p<0.05$ by Dunnett's multiple *t*-test.

Amine ratios (DOPAC/DA, HVA/DA, 5HIAA/5HT, and ACh/Ch) were calculated for each rat. There were no significant changes in monoamine contents and amine ratios in the frontal and occipital cortices of the BPA-treated groups. In the hippocampus, DA and DOPAC increased by 40 to 50% in the 40 mg/kg group compared to the control. These changes were not significant; however, the increase in Ch of the 4 mg/kg group was significant. Striatal Ch of the 4 mg/kg group was increased significantly compared to the control. Levels of Ch in the midbrain were high in BPA-treated groups and the ACh/Ch ratios of the 4 and 40 mg/kg groups were significantly smaller than those of the control. In the medulla oblongata, compared to the control the 5HT level of the 40 mg/kg group was low and Ch levels in the 4 and 40 mg/kg groups were high, however, none of these changes were statistically significant. The 5HIAA/5HT ratio was high in the 40 mg/kg group and the ACh/Ch ratio was low in the 4 mg/kg group, and these ratios significantly differed from the control. In the cerebellum, the DA content was high in the 40 mg/kg group (143% of the control), and DOPAC levels and DOPAC/DA ratios were low in the 4 and 40 mg/kg groups. Ch contents were

Table 1-1. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (frontal cortex, occipital cortex, and hippocampus)

		NE	DA	DOPAC	HVA	DOPAC/DA	HVA/DA
Frontal cortex	Control	100 ± 13.5	100 ± 15.6	100 ± 14.1	100 ± 5.0	100 ± 2.9	100 ± 16.4
	4 mg/kg	99.3 ± 4.0	90.3 ± 8.7	89.0 ± 4.8	89.3 ± 10.2	98.8 ± 6.0	90.0 ± 9.9
	40 mg/kg	90.7 ± 9.6	95.0 ± 10.0	90.2 ± 8.7	103.1 ± 5.0	94.5 ± 3.0	104.3 ± 15.5
	A100	1.75	6.31	1.39	0.779	0.222	0.137
Occipital cortex	Control	100 ± 9.5	100 ± 10.5	100 ± 8.9	100 ± 6.5	100 ± 3.1	100 ± 10.2
	4 mg/kg	94.1 ± 5.8	87.0 ± 4.8	78.0 ± 8.3	97.1 ± 7.1	88.2 ± 4.8	108.9 ± 9.1
	40 mg/kg	95.6 ± 4.6	83.4 ± 13.3	86.7 ± 10.4	99.6 ± 8.2	107.9 ± 11.4	123.6 ± 15.3
	A100	1.39	0.899	0.101	0.321	0.113	0.369
Hippocampus	Control	100 ± 5.2	100 ± 13.0	100 ± 4.8	100 ± 8.5	100 ± 9.5	100 ± 22.6
	4 mg/kg	122.1 ± 11.5	101.8 ± 10.9	88.0 ± 4.5	110.1 ± 5.9	84.5 ± 8.5	99.8 ± 13.5
	40 mg/kg	111.9 ± 8.6	141.5 ± 20.4	149.2 ± 28.9	109.0 ± 4.9	101.1 ± 7.8	73.6 ± 9.6
	A100	1.34	0.364	0.0291	0.389	0.0752	1.21

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5 for frontal and occipital cortex and N = 3–5 for hippocampus, nmoles/g tissue).

Table 1-2. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (frontal cortex, occipital cortex, and hippocampus)

		5HT	5HIAA	5HIAA/5HT	ACh	Ch	ACh/Ch
Frontal cortex	Control	100 ± 7.4	100 ± 12.0	100 ± 7.2	100 ± 12.9	100 ± 8.4	100 ± 10.1
	4 mg/kg	94.6 ± 2.9	93.8 ± 5.7	100.6 ± 6.3	106.8 ± 6.5	129.2 ± 8.6	84.5 ± 9.3
	40 mg/kg	107.3 ± 3.1	109.0 ± 5.5	102.7 ± 3.0	103.9 ± 16.1	126.4 ± 22.6	85.7 ± 10.2
	A100	7.76	0.918	0.117	10.1	11.9	0.848
Occipital cortex	Control	100 ± 5.5	100 ± 3.2	100 ± 8.2	100 ± 9.6	100 ± 17.4	100 ± 19.3
	4 mg/kg	103.9 ± 1.68	113.2 ± 7.2	107.4 ± 8.3	99.2 ± 4.4	129.1 ± 25.2	73.7 ± 11.3
	40 mg/kg	95.5 ± 10.5	103.4 ± 6.4	109.2 ± 7.4	90.3 ± 10.9	102.3 ± 18.9	85.3 ± 15.2
	A100	15.2	2.74	0.183	12.7	29.2	0.493
Hippocampus	Control	100 ± 5.3	100 ± 8.0	100 ± 5.1	100 ± 5.8	100 ± 16.4	100 ± 22.4
	4 mg/kg	109.1 ± 5.8	113.1 ± 13.4	102.8 ± 7.7	103.2 ± 13.4	155.5* ± 9.8	59.5 ± 10.3
	40 mg/kg	99.4 ± 11.7	88.6 ± 13.7	88.2 ± 7.0	108.2 ± 3.8	113.5 ± 10.4	86.4 ± 9.3
	A100	7.47	2.91	0.389	18.3	26.3	0.797

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5, nmoles/g tissue). *: $p < 0.05$ by Dunnett's multiple *t*-test.

high in the 4 and 40 mg/kg groups, however, none of these changes were statistically significant.

Effects of BPA on 9-wk-old offspring

Figures 3-1 to 3-4 show levels of neurotransmitters, metabolite, and metabolite/monoamine ratios at 9 wk after birth. The level of NE in the forebrain of the 40 mg/kg group was increased significantly compared with the control, although the increase was not great (123% of control). Compared to the control, the level of DA was unchanged, whereas that of DOPAC was significantly decreased (81% of control) in the forebrain of the 4 mg/kg group. The ratio of HVA/DA in the forebrain of the 40 mg/kg group was significantly low (73% of control). There were no significant changes in monoamine levels and metabolite/monoamine ratios in the hindbrain. Levels of DOPAC and 5HIAA were significantly decreased in the medulla oblongata of the 40 mg/kg group compared to the control. Ratios of DOPAC/DA and 5HIAA/5HT were lower than those of the control group, though differences were not significant. The concentration of DOPAC in the cerebellum was too low to detect consistently. Therefore, the DOPAC and DOPAC/DA data in the cerebellum were omitted from Fig. 3-4. Compared to the control, the 5HT and 5HIAA levels of the

Table 2-1. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (striatum, midbrain, and hypothalamus)

		NE	DA	DOPAC	HVA	DOPAC/DA	HVA/DA
Striatum	Control	100 ± 4.3	100 ± 8.4	100 ± 15.2	100 ± 12.4	100 ± 6.3	100 ± 6.1
	4 mg/kg	97.1 ± 15.5	95.9 ± 3.7	90.4 ± 5.7	93.4 ± 5.1	96.1 ± 5.4	98.1 ± 3.8
	40 mg/kg	96.4 ± 7.4	92.4 ± 4.2	87.0 ± 2.7	88.9 ± 4.4	96.5 ± 3.3	97.2 ± 4.5
	A100	1.01	32.0	2.94	3.57	0.090	0.111
Midbrain	Control	100 ± 15.8	100 ± 15.0	100 ± 14.4	100 ± 29.4	100 ± 2.9	100 ± 32.1
	4 mg/kg	102.5 ± 1.8	92.1 ± 10.6	91.0 ± 11.0	87.4 ± 15.0	99.2 ± 5.9	96.4 ± 22.8
	40 mg/kg	98.8 ± 6.5	101.9 ± 11.8	99.6 ± 12.0	74.2 ± 17.0	97.6 ± 2.7	76.4 ± 23.5
	A100	2.50	1.15	0.214	0.862	0.187	0.800
Hypothalamus	Control	100 ± 6.2	100 ± 6.7	100 ± 8.1	100 ± 26.2	100 ± 4.0	100 ± 27.0
	4 mg/kg	107.6 ± 6.7	105.0 ± 8.0	93.3 ± 8.0	120.0 ± 2.8	89.8 ± 7.7	111.5 ± 6.1
	40 mg/kg	97.1 ± 10.5	99.1 ± 7.9	93.1 ± 10.5	122.3 ± 10.6	93.6 ± 5.0	121.0 ± 13.1
	A100	6.50	2.68	0.440	1.43	0.164	0.555

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5, nmoles/g tissue).

Table 2-2. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (striatum, midbrain, and hypothalamus)

		5HT	5HIAA	5HIAA/5HT	ACh	Ch	ACh/Ch
Striatum	Control	100 ± 3.6	100 ± 7.9	100 ± 6.3	100 ± 6.4	100 ± 8.2	100 ± 7.9
	4 mg/kg	108.8 ± 8.7	98.9 ± 7.3	92.0 ± 6.0	110.9 ± 8.8	167.1* ± 18.6	68.7 ± 11.0
	40 mg/kg	104.4 ± 5.2	99.0 ± 4.2	96.2 ± 7.2	94.9 ± 5.2	121.7 ± 22.6	83.8 ± 11.6
	A100	3.25	3.33	1.02	36.2	20.3	1.82
Midbrain	Control	100 ± 9.7	100 ± 4.3	100 ± 10.4	100 ± 8.7	100 ± 18.5	100 ± 12.9
	4 mg/kg	85.8 ± 2.9	108.6 ± 9.8	122.4 ± 12.7	102.9 ± 6.8	178.8 ± 31.3	56.8* ± 10.0
	40 mg/kg	78.0 ± 5.3	110.6 ± 21.0	138.3 ± 27.9	91.3 ± 11.9	143.8 ± 31.1	62.7* ± 8.1
	A100	29.0	10.6	0.380	21.7	16.8	1.42
Hypothalamus	Control	100 ± 32.2	100 ± 37.4	100 ± 31.2	100 ± 18.9	100 ± 8.2	100 ± 11.5
	4 mg/kg	93.9 ± 6.9	80.3 ± 26.4	72.5 ± 21.8	100.1 ± 2.4	108.6 ± 8.4	95.4 ± 7.3
	40 mg/kg	115.8 ± 26.4	111.4 ± 38.4	79.1 ± 14.1	106.3 ± 13.0	128.1 ± 24.3	94.2 ± 17.2
	A100	18.2	10.2	0.648	16.5	11.0	1.47

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5, nmoles/g tissue). *: $p < 0.05$ by Dunnett's mutiple *t*-test.

cerebellum showed no changes in the BPA-treated groups, however, a significant difference was observed between the mean values of the 5HIAA/5HT ratio in the cerebellum of the control (1.356, 100%) and 40 mg/kg (1.083, 80%) groups.

Effects of BPA on the dams

Effects of BPA administration on the brain substances of dams are presented in Figs. 4-1 to 4-8. Variances in HVA and HVA/DA in the hippocampus, midbrain, and medulla oblongata were too large, therefore these data were not statistically analyzed. 5HT and 5HIAA were increased with statistical significance in the frontal cortex of the 4 mg/kg group, however, 5HIAA/5HT ratios in BPA-treated groups did not differ from those of the control. A tendency of increase in HVA and HVA/DA (229% of the control at 40 mg/kg) in the occipital cortex was observed in the BPA-treated groups, but these increases were not statistically significant when compared with the control. The level of DA was significantly increased in the hippocampus of the 4 mg/kg group. In the striatum, the level of 5HIAA was significantly increased in the 40 mg/kg group. No changes in monoamine contents and metabolite/monoamine ratios were observed in the midbrain, hypothalamus, medulla oblongata, and cerebellum of the BPA-treated groups.

Table 3-1. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (medulla oblongata and cerebellum)

		NE	DA	DOPAC	HVA	DOPAC/DA	HVA/DA
Medulla oblongata	Control	100 ± 5.9	100 ± 6.3	100 ± 5.0	100 ± 8.7	100 ± 6.1	100 ± 5.3
	4 mg/kg	96.1 ± 11.2	96.2 ± 11.9	88.8 ± 9.1	97.7 ± 8.2	92.2 ± 3.1	100.5 ± 13.9
	40 mg/kg	91.9 ± 3.2	105.6 ± 13.8	91.6 ± 13.2	96.2 ± 7.5	85.4 ± 3.8	92.0 ± 14.3
	A100	3.38	0.492	0.119	0.281	0.244	0.600
Cerebellum	Control	100 ± 4.4	100 ± 5.4	100 ± 18.2	100 ± 12.2	100 ± 19.1	100 ± 16.6
	4 mg/kg	104.4 ± 10.8	107.9 ± 10.4	69.3 ± 8.6	102.5 ± 7.4	64.7 ± 7.8	92.9 ± 4.5
	40 mg/kg	97.3 ± 3.5	142.5 ± 25.9	86.4 ± 16.6	118.0 ± 14.3	65.8 ± 7.8	92.9 ± 18.8
	A100	1.23	0.0636	0.0204	0.181	0.307	2.94

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5 for medulla oblongata and N = 3–5 for cerebellum, nmoles/g tissue).

Table 3-2. Effects of maternal administration of BPA on neurotransmitter contents of 6-wk-old female offspring (medulla oblongata and cerebellum)

		5HT	5HIAA	5HIAA/5HT	ACh	Ch	ACh/Ch
Medulla oblongata	Control	100 ± 12.5	100 ± 14.1	100 ± 9.8	100 ± 7.5	100 ± 17.8	100 ± 13.1
	4 mg/kg	115.3 ± 21.8	116.4 ± 11.1	121.2 ± 17.0	90.4 ± 8.6	182.0 ± 30.0	50.3* ± 10.8
	40 mg/kg	63.1 ± 5.2	95.3 ± 7.0	172.6* ± 18.3	90.8 ± 5.5	147.5 ± 34.6	68.1 ± 14.6
	A100	31.5	7.10	0.202	19.3	30.5	0.693
Cerebellum	Control	100 ± 29.3	100 ± 26.8	100 ± 25.1	100 ± 13.0	100 ± 21.3	100 ± 16.6
	4 mg/kg	90.4 ± 4.9	77.7 ± 17.9	86.3 ± 21.9	108.8 ± 16.9	152.0 ± 26.0	66.5 ± 11.6
	40 mg/kg	114.5 ± 13.9	103.9 ± 15.3	93.3 ± 15.7	97.6 ± 14.3	128.8 ± 17.0	70.9 ± 13.1
	A100	1.35	0.251	0.188	3.47	12.3	0.326

Results are shown as means ± SEM (%). A100, Absolute values for 100% (N = 4–5, nmoles/g tissue). *: $p < 0.05$ by Dunnett's mutiple *t*-test.

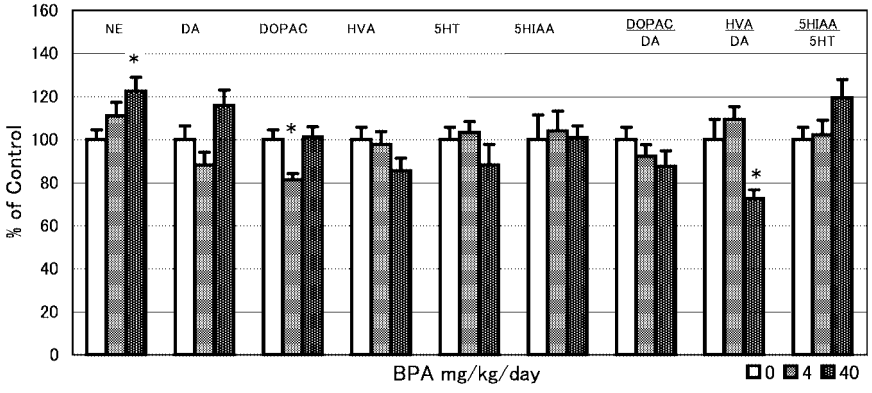

Fig. 3-1. Effects of perinatal administration of BPA on the neurotransmitter contents of forebrain in 9-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.722 for NE, 6.44 for DA, 0.945 for DOPAC, 1.32 for HVA, 12.0 for 5HT, and 1.35 for 5HIAA; absolute values of ratios for 100% were as follows: 0.148 for DOPAC/DA, 0.210 for HVA/DA, and 0.111 for 5HIAA/5HT. N = 5. *: $p<0.05$ by Dunnett's multiple *t*-test.

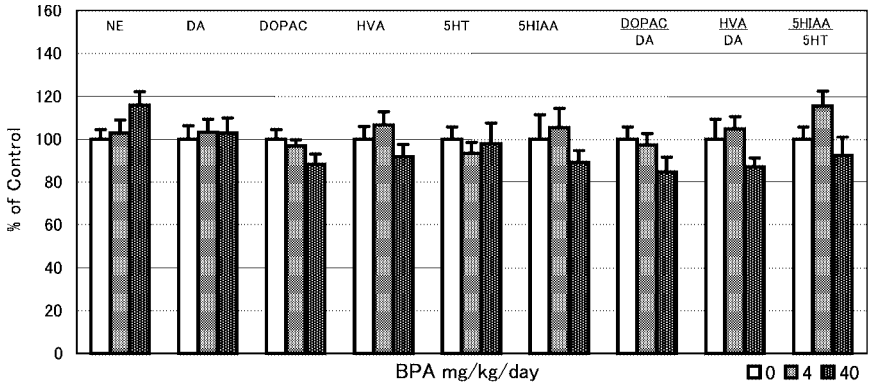

Fig. 3-2. Effects of perinatal administration of BPA on the neurotransmitter contents of hindbrain in 9-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.65 for NE, 1.64 for DA, 0.203 for DOPAC, 0.570 for HVA, 9.58 for 5HT, and 3.87 for 5HIAA; absolute values of ratios for 100% were as follows: 0.126 for DOPAC/DA, 0.361 for HVA/DA, and 0.420 for 5HIAA/5HT. N = 5.

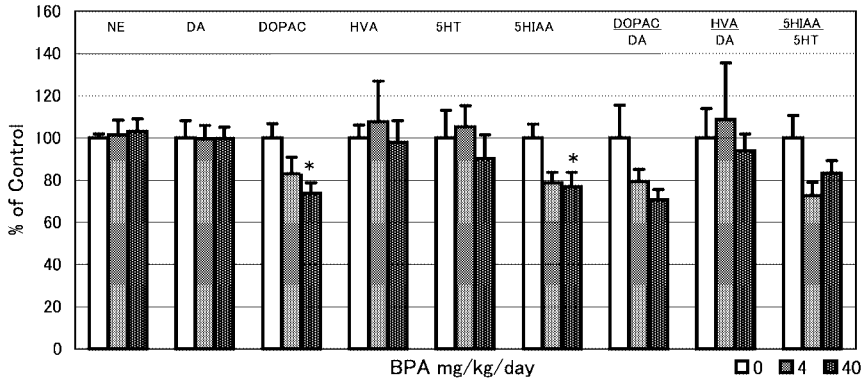

Fig. 3-3. Effects of perinatal administration of BPA on the neurotransmitter contents of medulla oblongata in 9-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.98 for NE, 0.193 for DA, 0.0881 for DOPAC, 0.695 for HVA, 2.47 for 5HT, and 3.30 for 5HIAA; absolute values of ratios for 100% were as follows: 0.479 for DOPAC/DA, 3.76 for HVA/DA, and 1.41 for 5HIAA/5HT. N = 5. *: $p<0.05$ by Dunnett's multiple *t*-test.

Discussion

The reproductive effects of BPA have been studied in detail[23], but little is understood about the effects of BPA on the nervous system. Both positive and negative effects of BPA on the reproductive and other functions of offspring after perinatal exposure have been reported[7, 10, 24, 25]. In our present study, changes in monoamine and metabolite levels due to BPA treatment were not observed in the brains of 1-wk-old female rat pups. Increases in DA metabolite, DOPAC and HVA, were observed in the female rat pups at 3 wk of age, and these increases were statistically significant in the forebrain, DOPAC in the 40 mg/kg group and HVA in the 4 mg/kg group. HVA contents in the hindbrain and medulla oblongata of the 40 mg/kg group were greater than the control. The HVA/DA ratio was significantly high in the frontal cortex of the 4 mg/kg group and it was also high in the occipital cortex and medulla oblongata of the 40 mg/kg group, but the differences were not significant. These results mean that the turnover of DA was accelerated in the BPA-treated groups, and suggest that the release of DA from nerve endings was increased in these groups. Significant increases in 5HT and 5HIAA were observed in the forebrain, and in 5HT in the medulla oblongata of the 4 mg/kg group. The 5HT level

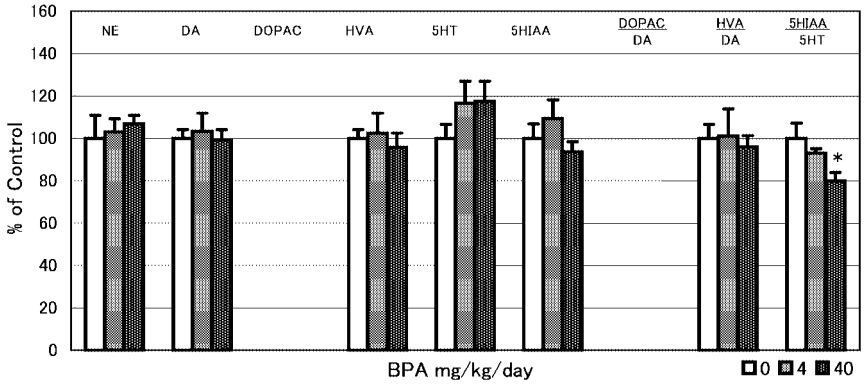

Fig. 3-4. Effects of perinatal administration of BPA on the neurotransmitter contents of cerebellum in 9-wk-old female offspring.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.635 for NE, 0.0355 for DA, 0.411 for HVA, 0.346 for 5HT, and 0.464 for 5HIAA; absolute values of ratios for 100% were as follows: 11.7 for HVA/DA, and 1.36 for 5HIAA/5HT. N = 5. *: $p<0.05$ by Dunnett's multiple *t*-test.

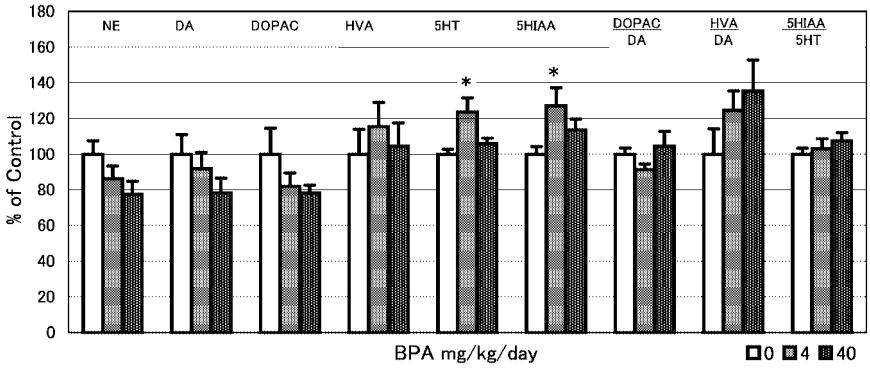

Fig. 4-1. Effects of perinatal administration of BPA on the neurotransmitter contents of frontal cortex in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.65 for NE, 4.74 for DA, 1.11 for DOPAC, 0.771 for HVA, 13.8 for 5HT, and 1.70 for 5HIAA; absolute values of ratios for 100% were as follows: 0.232 for DOPAC/DA, 0.166 for HVA/DA, and 0.123 for 5HIAA/5HT. N = 5–6. *: $p<0.05$ by Dunnett's multiple *t*-test.

in the medulla oblongata of the 40 mg/kg group was higher than the control; however, no changes were observed in 5HT and 5HIAA in the forebrain of the 40 mg/kg group. The 5HT and 5HIAA levels in the hindbrain of the BPA-treated groups were less than control, although the degree of the decrease was small. Though 5HT and 5HIAA increased in the forebrain and medulla oblongata, changes in 5HT and 5HIAA seemed to be dependent on brain area and dose of BPA. In 6-wk-old offspring, increases in Ch levels were observed in all of the eight brain areas of the BPA-treated groups, but there were no accompanying changes in ACh levels. Synthesis or uptake into the synaptosome of Ch seems to have been accelerated in the BPA-treated groups. Among changes in catecholamine, serotonin, and their metabolites, DA and DOPAC in the hippocampus, and DA in the cerebellum increased in the 40 mg/kg group by 40 to 50% compared with the control. In 9-wk-old offspring, significant changes in monoamines and metabolites were observed in the forebrain, medulla oblongata, and cerebellum of the BPA-treated groups, however, these changes were scattered and not great.

BPA treatment affected the monoamine and metabolite contents of the brain of dams. Large and dose dependent increases in HVA and in the HVA/DA ratio occurred in the

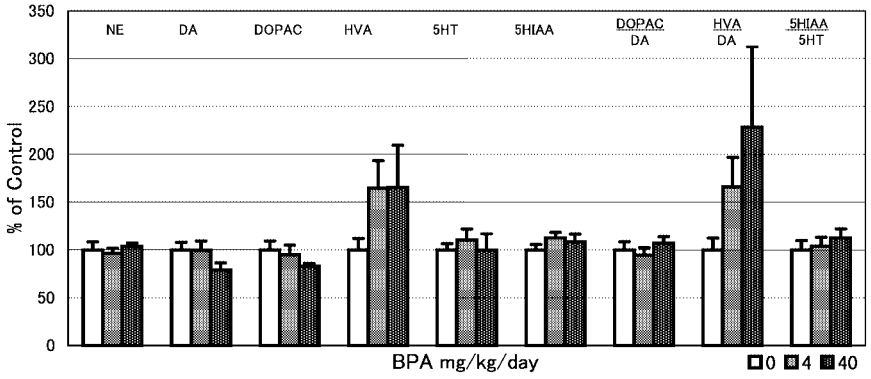

Fig. 4-2. Effects of perinatal administration of BPA on the neurotransmitter contents of occipital cortex in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.59 for NE, 3.44 for DA, 0.609 for DOPAC, 0.0749 for HVA, 2.83 for 5HT, and 1.59 for 5HIAA; absolute values of ratios for 100% were as follows: 0.179 for DOPAC/DA, 0.0221 for HVA/DA, and 0.576 for 5HIAA/5HT. N = 5–6.

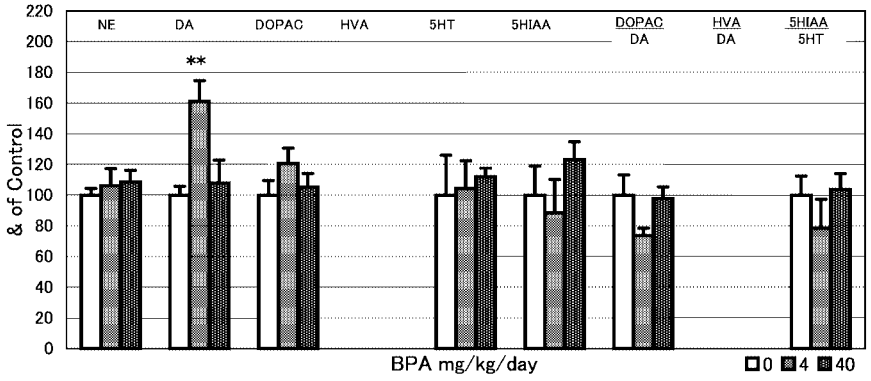

Fig. 4-3. Effects of perinatal administration of BPA on the neurotransmitter contents of hippocampus in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.37 for NE, 0.123 for DA, 0.0673 for DOPAC, 2.06 for 5HT, and 1.97 for 5HIAA; absolute values of ratios for 100% were as follows: 0.564 for DOPAC/DA, and 1.02 for 5HIAA/5HT. N = 5–6. **: $p<0.01$ by Dunnett's multiple *t*-test.

occipital cortex of the BPA-treated groups, although they were not statistically significant. A dose dependent increase in the HVA/DA ratio was also observed in the frontal cortex. These findings suggest that DA turnover was accelerated in specific brain areas following BPA treatment. 5HT and 5HIAA increased in the frontal cortex of the 4 mg/kg group and 5HIAA increased in the striatum of the 40 mg/kg group. Everitt *et al.* reported that the serotonin turnover was accelerated in female rats by estrogen administration[26]. According to Shimizu and Bray, estradiol administration increased the ratio of DOPAC/DA but decreased the 5HIAA/5HT ratio in the nucleus accumbens of ovariectomized rats when measured by microdialysis[27]. Our findings are consistent with the changes found by Everitt *et al.* following estrogen treatment, because 5HIAA in the frontal cortex and striatum increased in BPA-treated dams. BPA possesses very weak estrogenic activity and the 5HIAA increase observed in our experiment may be related to the estrogenic activity of BPA. Our experimental conditions were very different from those of Shimizu and Bray, therefore, it is difficult to compare our findings with their results. They measured extracellular neurotransmitters and metabolites in a microdialysis study, whereas, we measured them following the homogenization of the brain, in which both intracellular

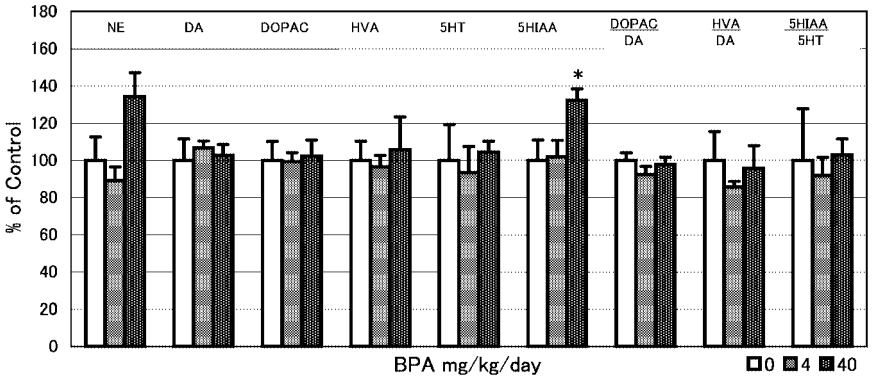

Fig. 4-4. Effects of perinatal administration of BPA on the neurotransmitter contents of striatum in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.669 for NE, 48.2 for DA, 7.16 for DOPAC, 3.75 for HVA, 1.39 for 5HT, and 2.05 for 5HIAA; absolute values of ratios for 100% were as follows: 0.150 for DOPAC/DA, 0.0819 for HVA/DA, and 1.85 for 5HIAA/5HT. N = 5–6. *: $p<0.05$ by Dunnett's multiple *t*-test.

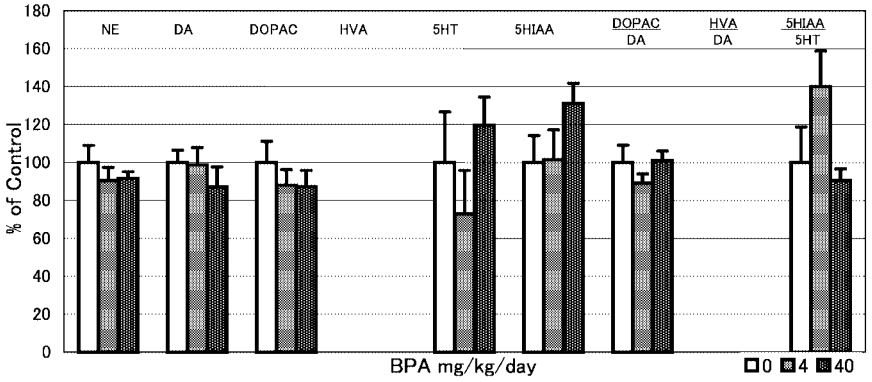

Fig. 4-5. Effects of perinatal administration of BPA on the neurotransmitter contents of midbrain in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 6.87 for NE, 4.75 for DA, 1.44 for DOPAC, 3.11 for 5HT, and 3.54 for 5HIAA; absolute values of ratios for 100% were as follows: 0.304 for DOPAC/DA, and 1.41 for 5HIAA/5HT. N = 4–6.

and extracellular substances were included. At present, it is not clear whether the changes in monoamine turnover observed in the dams in our experiments were due to the estrogenic activity of BPA. An effect of BPA on prolactin secretion has been reported[28, 29]. DA inhibits the secretion of prolactin in the anterior pituitary gland. Male rats were exposed to BPA from postnatal days 22 to 32[29]. During this period, BPA stimulated prolactin secretion in the same manner as pimozide (a dopamine antagonist) and 17β-estradiol. Steinmetz *et al.* reported that BPA induces hyperprolactinemia in F344 rats with an efficacy similar to that of estradiol[28]. On the assumption that such effects of BPA on prolactin secretion are via the inhibition of dopaminergic activity in the anterior pituitary gland, BPA would inhibit the activity of DA neurons. In our experiment, DA in the hippocampus significantly increased in the 4 mg/kg group. HVA levels and HVA/DA ratios in the occipital cortex of dams treated with BPA at 4 or 40 mg/kg were higher than the control, although without statistical significance. These results from female CD (SD) IGS rats are inconsistent with previous findings. This may be due to sex differences. Alternatively, the stimulation of prolactin secretion by BPA might be due to activities of BPA other than dopamine-mediated action. Changes in DA, 5HT, and their metabolites were observed in some brain regions of dams dosed with 4 or 40 mg/kg of BPA in our present study. Although the

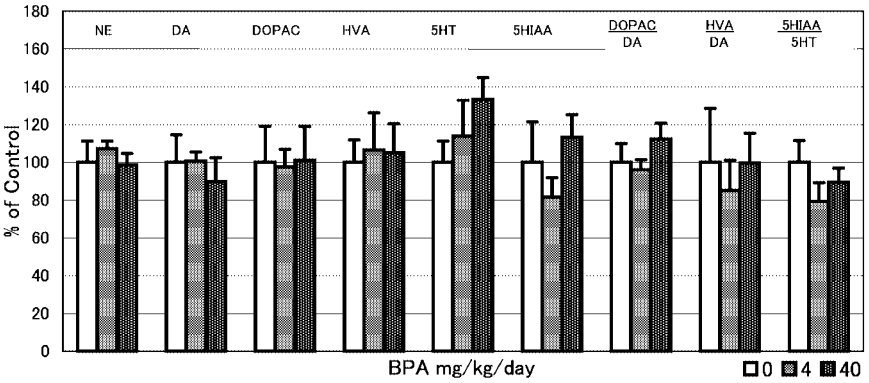

Fig. 4-6. Effects of perinatal administration of BPA on the neurotransmitter contents of hypothalamus in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 9.56 for NE, 2.07 for DA, 0.498 for DOPAC, 0.192 for HVA, 4.91 for 5HT, and 2.55 for 5HIAA; absolute values of ratios for 100% were as follows: 0.240 for DOPAC/DA, 0.113 for HVA/DA, and 0.497 for 5HIAA/5HT. N = 4–6.

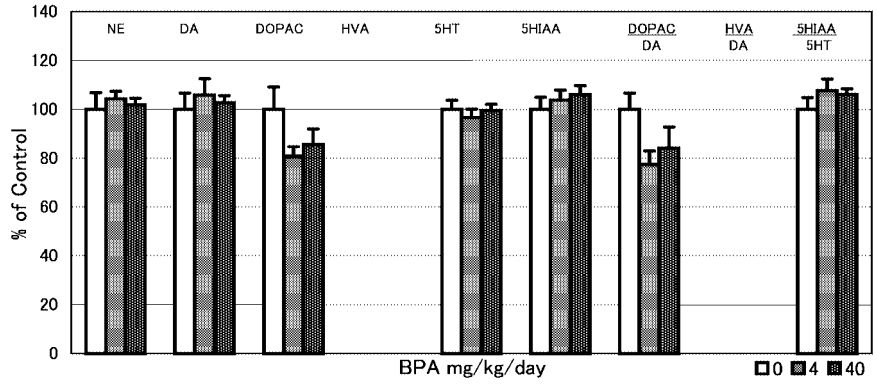

Fig. 4-7. Effects of perinatal administration of BPA on the neurotransmitter contents of medulla oblongata in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 2.64 for NE, 0.463 for DA, 0.232 for DOPAC, 2.36 for 5HT, and 2.62 for 5HIAA; absolute values of ratios for 100% were as follows: 0.502 for DOPAC/DA, and 1.11 for 5HIAA/5HT. N = 5–6.

effects of BPA on GABA (A) and nicotinic receptors have been reported[30, 31], those of BPA on dopaminergic and serotonergic neurons have not been described. Our results suggest that the metabolism of DA and 5HT was accelerated in BPA-treated female rats. We postulate that BPA may affect some DA- and 5HT-related brain functions.

The assay of brain substances of the male offspring sacrificed in the same series of experiments is now underway in our laboratory. Kubo *et al.* reported that sexual differentiation of the brain locus coeruleus is disrupted in rats perinatally exposed to BPA[32]. In that study, maternal rats received BPA at 1.5 mg/kg per day. We found that levels of NE in the forebrain of 9-wk-old offspring were dose-dependently increased and reached significance in the 40 mg/kg group; a similar increase was also observed in the hindbrain. Cell bodies of NE neurons are dense in the locus coeruleus and NE neurons might be altered by BPA in this disruption of sexual differentiation, although the size of the locus coeruleus is much smaller than the forebrain and hindbrain. According to Farabollini *et al.*, the maternal administration of BPA during the critical period of fetal brain organization produces different effects on the behavior of male and female offspring rats[33]. A comparison of data regarding neurotransmitters obtained from female and male offspring may explain such sexually differentiated behavior effects of BPA.

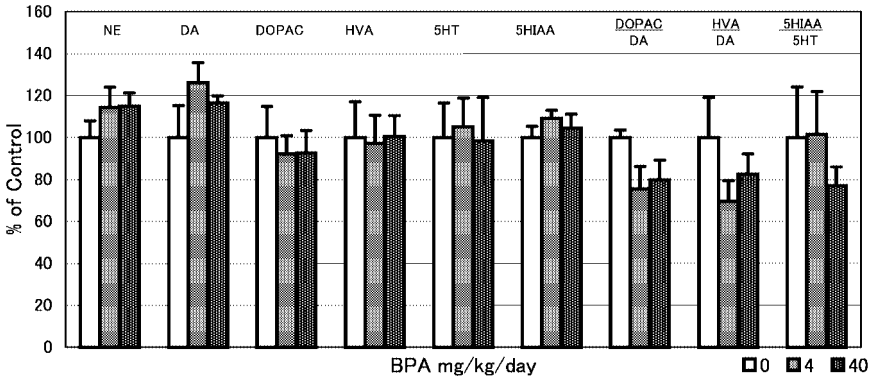

Fig. 4-8. Effects of perinatal administration of BPA on the neurotransmitter contents of cerebellum in maternal rats.

Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 0.785 for NE, 0.0527 for DA, 0.0520 for DOPAC, 0.0447 for HVA, 0.455 for 5HT, and 0.366 for 5HIAA; absolute values of ratios for 100% were as follows: 0.987 for DOPAC/DA, 0.896 for HVA/DA, and 0.939 for 5HIAA/5HT. N = 5–6.

We found that dams given BPA at 400 mg/kg weighed significantly less than controls[12]. The 40 mg/kg group weighed somewhat less than controls, but BPA at 4 mg/kg did not affect the body weight of dams. The weight of the 40 mg/kg group recovered to the control level during lactation. The body weight of female offspring did not statistically differ between control and BPA-treated groups at 1 to 9 wk of age. No differences were statistically significant in the weights of the liver and kidneys among groups. These results show that the neurochemical alterations in the brains of dams and offspring after BPA exposure were not caused by differences in somatic growth. Anogenital distances in female offspring were not significantly affected by BPA at 1, 3 or 9 wk of age as observed in the same rats in this study[12]. Though the anogenital distance is not always sensitive to the reproductive effects of chemicals, neurotransmitters in the brain might be more sensitive to BPA than reproductive organ sensitivity to the estrogenic action of BPA. At present we have no data to explain the reason why the changes in monoamines and metabolites occurred in pups as well as dams. These changes were observed in specific brain areas, except the increase in Ch. Levels of Ch were higher than control in all of the eight brain areas of 6-wk-old pups of the 4 and 40 mg/kg groups. Unfortunately, we have no data for Ch in pups at ages other than 6 wk.

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