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.

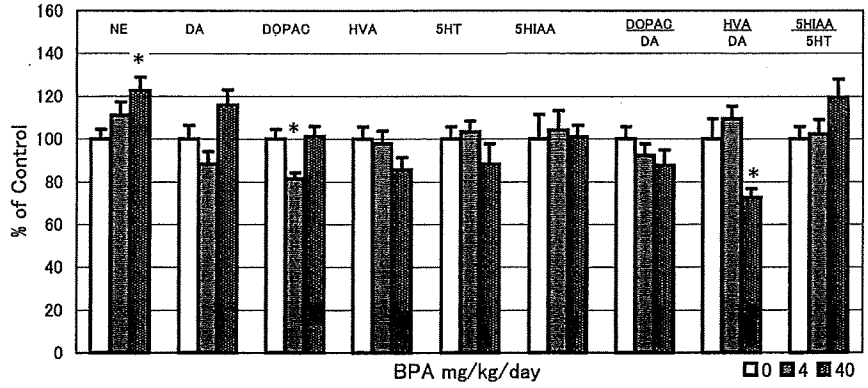

Fig. 3-1. Effects of perinatal administration of BPA on the neurotransmitter contents of forebrain in 9-wk-old female offspring.

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.

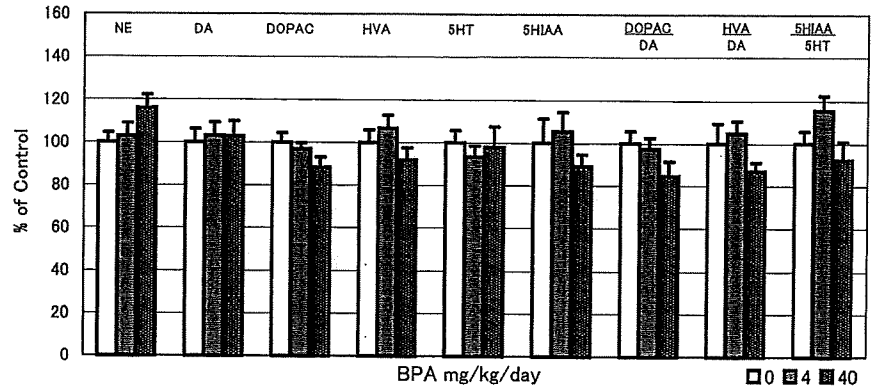

Fig. 3-2. Effects of perinatal administration of BPA on the neurotransmitter contents of hindbrain in 9-wk-old female offspring.
Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.65 for NE, 1.64 for DA, 0.203 for DOPAC, 0.570 for HVA, 9.58 for 5HT, and 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.

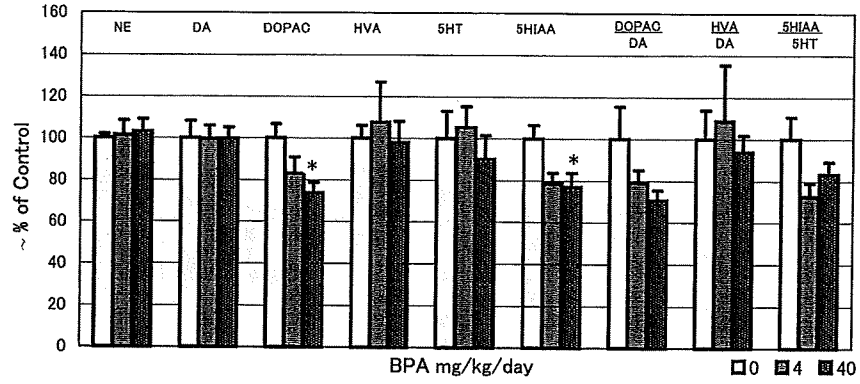

Fig. 3-3. Effects of perinatal administration of BPA on the neurotransmitter contents of medulla oblongata in 9-wk-old female offspring.
Results are shown as means ± SEM (%). Absolute values (nmoles/g tissue) for 100% were as follows: 1.98 for NE, 0.193 for DA, 0.0881 for DOPAC, 0.695 for HVA, 2.47 for 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

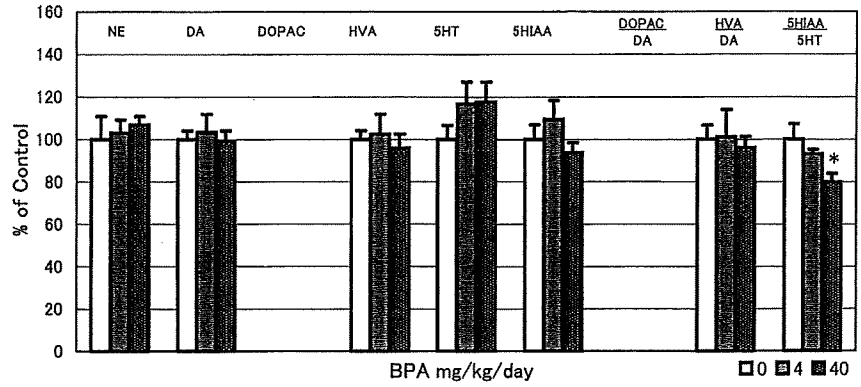

Fig. 3-4. Effects of perinatal administration of BPA on the neurotransmitter contents of cerebellum in 9-wk-old female offspring.

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.

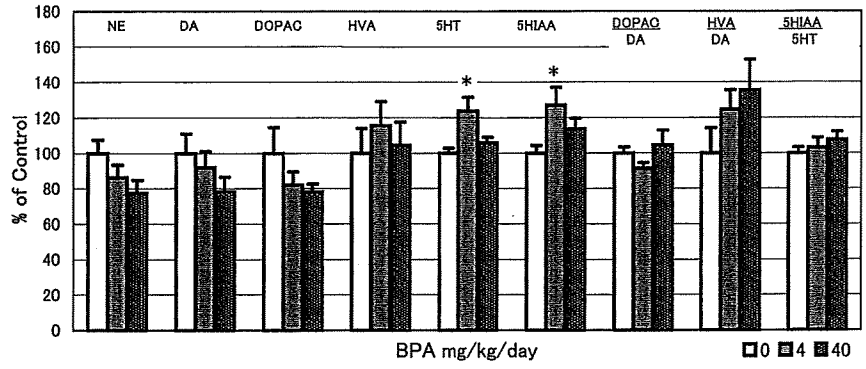

Fig. 4-1. Effects of perinatal administration of BPA on the neurotransmitter contents of frontal cortex in maternal rats.

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

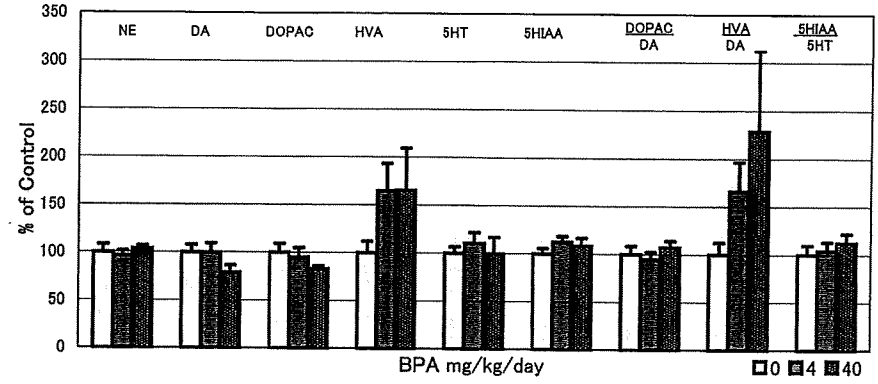

Fig. 4-2. Effects of perinatal administration of BPA on the neurotransmitter contents of occipital cortex in maternal rats.

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.

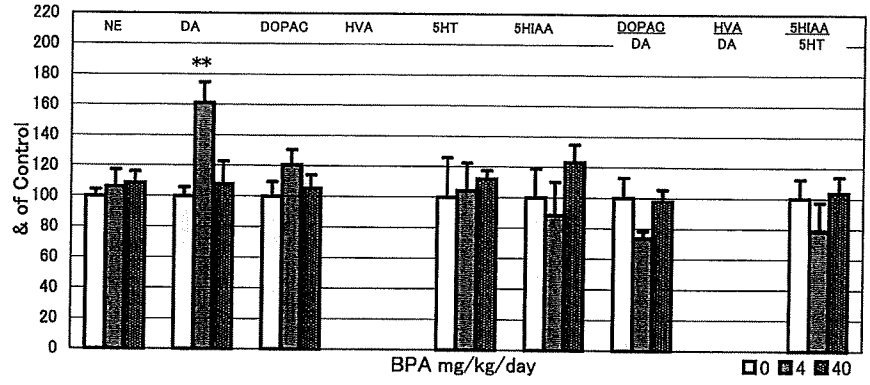

Fig. 4-3. Effects of perinatal administration of BPA on the neurotransmitter contents of hippocampus in maternal rats.

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

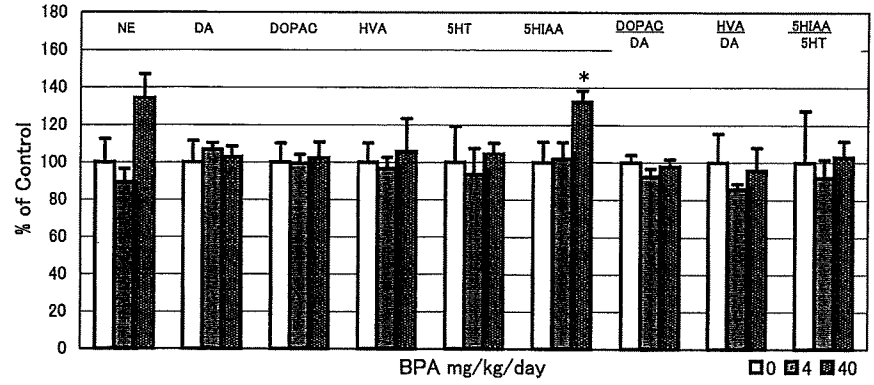

Fig. 4-4. Effects of perinatal administration of BPA on the neurotransmitter contents of striatum in maternal rats.

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.

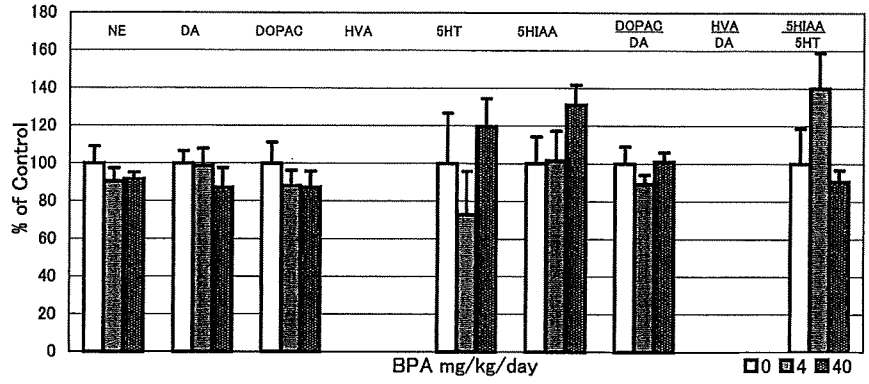

Fig. 4-5. Effects of perinatal administration of BPA on the neurotransmitter contents of midbrain in maternal rats.

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

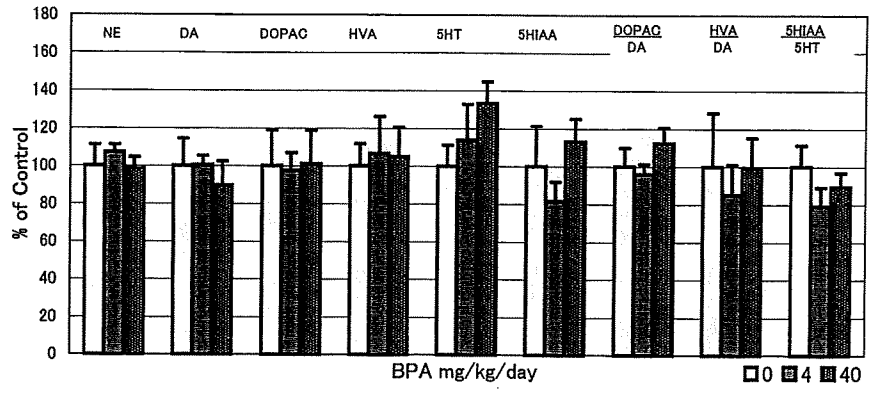

Fig. 4-6. Effects of perinatal administration of BPA on the neurotransmitter contents of hypothalamus in maternal rats.

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.

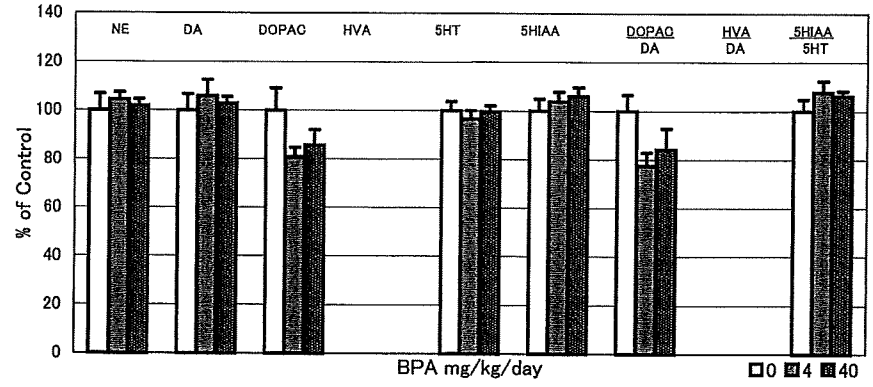

Fig. 4-7. Effects of perinatal administration of BPA on the neurotransmitter contents of medulla oblongata in maternal rats.

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.

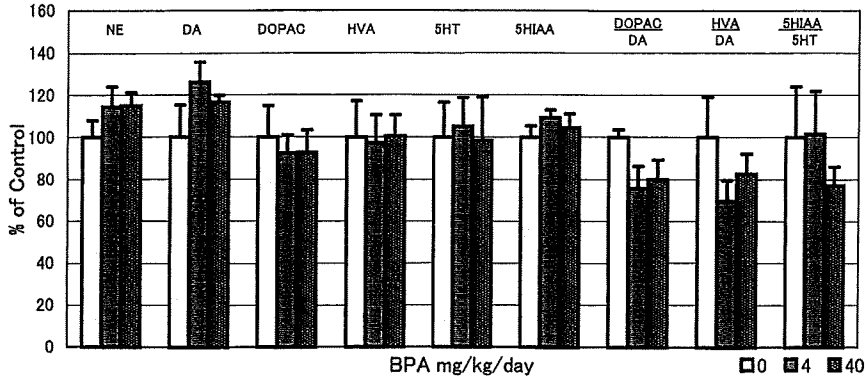

Fig. 4-8. Effects of perinatal administration of BPA on the neurotransmitter contents of cerebellum in maternal rats.

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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Original Article

Effects of in Utero and Lactational Exposure to Di(2-ethylhexyl)phthalate on Somatic and Physical Development in Rat Offspring

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Abstract: Di(2-ethylhexyl)phthalate (DEHP) has been reported to act as an antiandrogen and to affect the reproductive organs and accessory genital glands. Thus, to assess the reproductive toxicity of DEHP it is important to examine both its adverse effects on the development of offspring following maternal exposure and its effects on sexual function and fertility. In the present study, we examined whether in utero and lactational exposure to DEHP affects postnatal somatic growth of offspring in the rat. Pregnant females were orally administered various doses of DEHP (0, 25, 100 or 400 mg/kg body weight/day) from gestational day (GD) 6 through postnatal day (PND) 20. There were no significant changes in body weight, body length, tail length, or the weight of individual organs between the control and DEHP-treated groups. Somatic hormonal parameters were the same for all DEHP doses. These findings suggest that in utero and lactational exposure to various concentrations of DEHP has very little effect on postnatal development or endocrine and physical status of male and female rat offspring under the experimental conditions of the present study.

Key words: Di(2-ethylhexyl)phthalate, Postnatal development, In utero and lactational exposure, Offspring, Rat

Introduction

To date, several compounds have been suspected of exerting endocrine-disturbing effects even at ultra-low concentrations. Phthalates have been produced and used in the manufacture of chemically derived materials and products. Di(2-ethylhexyl)phthalate (DEHP) has been most widely used in polyvinyl chloride to impart structural flexibility, and it is used as a plasticizer in products such as food packaging, children's products (toys and crib bumpers) and medical devices. Significantly, DEHP has been detected in plasma samples[1]. Mono(2-ethylhexyl)phthalate (MEHP), which is an active and the predominant DEHP metabolite, is also considered as a testicular toxicant[2]. It has been estimated that mean DEHP intake is 8.2 μg/kg body weight per day for adults[3]. During recent years, DEHP has been excluded from many products to avoid consumer exposure. However, recent heightened public concerns about environmental exposure to high concentrations of DEHP have raised new questions about its possible occupational and medical health hazards.

Developmental toxicity studies of DEHP have been conducted in laboratory mice[4–8] and rats[8–10]. These reports suggest that in utero exposure to high doses of DEHP induces embryotoxicity and/or teratogenicity. Animal reproductive toxicity studies of DEHP have also been reported. In a study of adult male rats, testicular defects such as atrophy of the seminiferous tubules, loss of spermatogenesis and vacuolation of Sertoli cells were observed after 90 days of dietary exposure to DEHP at 500 and 5,000 ppm (equivalent to 37.6 and 375.2 mg/kg/day, respectively)[11]. Perinatal exposure to DEHP in rats from gestational day (GD) 14 through postnatal day (PND) 3 reduced anogenital distance, testis weight or the weight of androgen-dependent tissues[12].

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Dietary exposure of adult male rats given 0, 320, 1,250, 5,000, and 20,000 ppm DEHP (equivalent to 0, 17.5, 69.2, 284.1 and 1156.4 mg/kg/day, respectively) for 60 days, when mated with untreated adult females, did not affect the rate of neonatal death, initial pup weight or growth (up to PND 7), whereas the average litter size decreased in rats fed 20,000 ppm DEHP[13]. Inhalation exposure of adult male Wistar rats to 25 mg/m^3 for 6 h/day for 8 wk increased plasma testosterone level and seminal vesicle weight in a dose-dependent manner[14]. In a study of adult female rats, DEHP induced prolonged estrous cycles and suppressed plasma concentrations of estradiol and subsequent ovulation[15].

Several studies have shown that in utero and lactational exposure to DEHP leads to abnormalities in the hypothalamus-pituitary-testicular axis. Sprague-Dawley rats were orally dosed with DEHP (0–1,500 mg/kg/day) from GD 3 through PND 21, and dose-related effects in the male offspring included several parameters involved in sexual development[16]. Oral exposure of pregnant female Long-Evans rats to 100 mg/kg/day DEHP from GD 12–21 induced significantly increased levels of testosterone and luteinizing hormone in male offspring on PND 21 and PND 35, but by PND 90 the levels were comparable between treated and untreated animals[17], indicating that the magnitude of DEHP toxicity on reproductive function is influenced by the stage of development.

Thus, DEHP toxicity studies in laboratory animals have focused on embryotoxicity, teratogenicity and reproductive toxicological effects in addition to some developmental effects in the early postnatal period, yet extensive toxicity information for long-term development after DEHP exposure is still lacking. The purpose of the present study was to evaluate postnatal growth and physical development following in utero and lactational exposure to DEHP in male and female rat offspring until the post-pubertal period. We examined the effects of DEHP on pubertal development, and doses of DEHP were chosen based on the levels that caused no overt maternal toxicity. Additionally, the exposure period was extended to examine the effects of lactational exposure in addition to the effects of in utero exposure, to complement previous studies[4–10]. Thus, we administered several doses of DEHP orally by gavage to pregnant rats using an experimental schedule identical to one used previously[18], and we examined the effects on postnatal somatic and organ growth, as assessed by body weight, body length, tail length and main organ weights, including reproductive organs, in male and female offspring. In addition, to better assess physical status following DEHP exposure, we evaluated the levels of several plasma hormonal landmarks with regard to postnatal somatic growth.

Materials and Methods

Chemicals and experimental animals

DEHP (purity >99.9%, Cat# 289-10442) and corn oil were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. A total of 52 pregnant (GD 3) female rats (Crj: CD (SD) IGS strain, 9 wk of age) were purchased from Charles River Japan, Inc. (Tsukuba, Japan). The presence of a copulatory plug defined GD 0. They were acclimated on GD 3–6 and housed individually in plastic cages with sterilized wood chips (Soft chip, Japan Slc Inc., Shizuoka, Japan) for bedding and were maintained under controlled temperature (23 ± 1°C) and humidity (55 ± 5%) and with a 12-h light-dark cycle (08:00–20:00) throughout the study. A standard laboratory diet (CE-2, Clea Japan, Inc., Tokyo, Japan) and drinking water were available ad libitum.

Dose range-finding evaluation

Dams were randomly divided into five groups (four pregnant females per group). The DEHP-exposed groups were orally administered 500, 1,000, 1,500, or 2,000 mg DEHP/kg/day in corn oil vehicle (10 ml/kg of body weight); DEHP was given between 08:30 and 09:30 for five consecutive days each week (Monday–Friday) from GD 6 through GD 20, and the control group was given the same amount of corn oil during the same period. During the exposure period, we recorded maternal body weights and noted any clinical signs or abnormal behavior that may have resulted from toxic effects. These results were used to determine the range of the DEHP dose for the main study.

Main study

Dams were randomly divided into four groups (eight pregnant females per group) and weighed once daily from GD 3 through PND 20 (except for GD 4 and 5). The DEHP-exposed groups were orally administered 25, 100 or 400 mg DEHP/kg/day in corn oil vehicle (10 ml/kg of body weight); DEHP was given between 08:30 and 09:30 from GD 6 through PND 20, and the control group was given the same amount of corn oil during the same period. Maternal data were recorded as described above. For each dam, the gestational duration was recorded, and weight gain during gestation and lactation was measured. Dams were checked for birth until 10:00 on each day; the day on which pups were first observed was designated PND 0. The number of

live births and the weight of each live pup on PND 1 were recorded. The litter size was standardized to 10 (five males and five females when possible) between 10:00 and 11:00 on PND 7 (1 wk of age). Litters with a total of nine or fewer pups were not culled regardless of the sex ratio. Culled pups were used for the analysis at 1 wk of age. On PND 21, the remaining offspring were weaned, and thereafter males and females were housed in separate stainless steel cages by litter. Body weights were recorded with an electric balance (Shimadzu, Kyoto, Japan). Body length and tail length (millimeters) were measured with a digital caliper (Mitutoyo, Kanagawa, Japan). The nose-anus length was considered the body length. One male and one female offspring from each dam were dissected at 3 and 9 wk of age when possible. While the rat was under ether anesthesia, liver, kidneys and testes, prostate and seminal vesicles or ovaries and uterus were carefully removed and weighed.

Hormone determinations

For hormone determinations, blood samples were collected from the postcaval vein following euthanasia by ether inhalation at 9 wk of age. Plasma samples were obtained by centrifugation at 4°C and stored at –20°C until the analysis. Concentrations of the plasma thyroid hormones thyroxine (T_4) and tri-iodothyronine (T_3) were determined by a time-resolved fluoroimmunoassay (DELFIA T_4 Reagents and DELFIA T_3 Reagents, respectively, PerkinElmer Life and Analytical Sciences, Inc., MA, USA). Plasma growth hormone (GH) concentrations were determined by enzyme immunoassay (EIA) (Rat GH EIA Biotrak system, GE Healthcare Bio-Sciences Corp., NJ, USA). Plasma insulin-like growth factor-I (IGF-I) concentrations were also measured by EIA (ACTIVE mouse/rat IGF-I EIA kit, Diagnostic Systems Laboratories, Inc., TX, USA). Time-resolved fluorescence and absorbance were measured by a multilabel counter (VICTOR^2, PerkinElmer Life and Analytical Sciences, Inc.). All hormones were assayed according to the manufacturer's instructions.

Statistical analysis

The differences from the corresponding control group were statistically analyzed by an analysis of variance followed by Dunnett's test (significance at $p<0.05$).

Results

Dose range-finding evaluation

In the 1,000 mg/kg/day and higher DEHP groups, maternal toxicity was clearly manifested as greatly suppressed weight gain during gestation, which led us to discontinue subsequent dosing by GD 17 of this preliminary study. In the 500 mg/kg/day group, mean body weights decreased slightly at later stages of gestation compared with the control group (data not shown). Based on these observations, we set the highest dose at 400 mg/kg/day to exclude the influence of maternal toxicity and observe the effect of DEHP on the offspring. The lowest dose and the middle dose were set at 25 mg/kg/day and 100 mg/kg/day, respectively.

Main study

Dams

Table 1 shows the number of dams and their offspring used for examinations in each group. Weight gain did not differ between dams from the control group and the DEHP groups from GD 6 through GD 21. In the 400 mg/kg/day group, one dam was found dead on GD 23, and thus the dam was excluded from the analysis. No significant differences were observed between the control group and the DEHP groups with regard to gestational duration or the number of live births per litter on PND 1.

Figure 1 shows maternal body changes during gestation (left panel) and lactation (right panel). There were no statistically significant differences among groups with regard to maternal body weight during the gestation and lactation periods, although the 25 mg/kg/day group showed a transient but not significant weight reduction during early lactation.

Offspring

The number of offspring examined is shown in Table 2. In male and female offspring, there were no statistically significant differences in body weight, body length or tail length between the control and DEHP-exposed groups at 1, 3 or 9 wk of age (Figs. 2, 3 and 4). There were no statistically significant effects on liver or kidney weights in males or females at 1, 3 or 9 wk of age (Table 3, 4). In male offspring, testis weights did not differ among the control group and DEHP groups at 3 or 9 wk of age (Table 3). Prostate and seminal vesicle weights did not differ among the control group and DEHP groups at 9 wk of age (Table 3). In female offspring, ovary and uterus weights did not differ among the groups at 3 or 9 wk of age (Table 4).

Physical status of offspring

In male offspring, no statistically significant differences in plasma concentrations of T_4, T_3, GH or IGF-I were observed among the control group and the DEHP groups at 9 wk of age (Table 5). In female offspring, no statistically significant differences in plasma concentrations of T_4, T_3,

Table 1. Dams and litter data

	DEHP dose (mg/kg/day)			
	0	25	100	400
Females (n)	8	8	8	8
Pregnant females (n)	8	8	8	8
Dam weight gain (GD 6-21)	130 ± 7[a]	127 ± 5	135 ± 4	133 ± 5
Gestational period (days)	21.1 ± 0.1	21.4 ± 0.2	21.3 ± 0.2	21.3 ± 0.2
Live births/litter on PND 1	11.8 ± 0.7	13.6 ± 0.6	13.5 ± 0.5	11.7 ± 0.5 (7)[b]

[a]Values are mean ± SEM.

[b]The number in parentheses represents dams per dose group. One dam was found dead on GD 23, and thus the dam was excluded from the analysis.

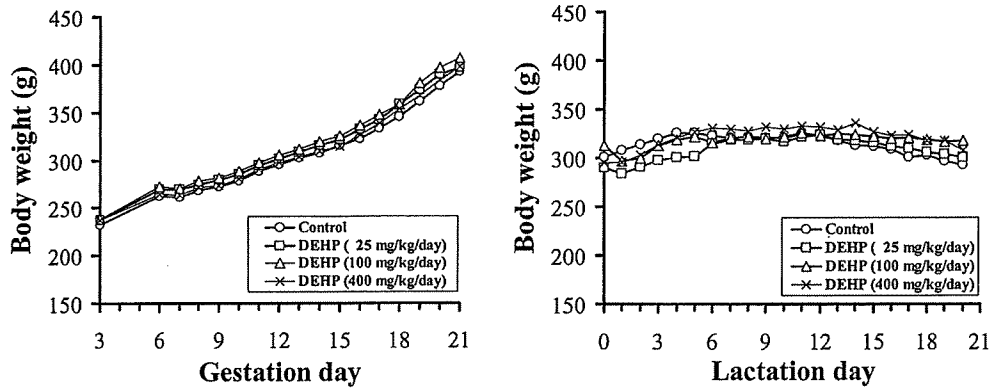

Fig. 1. Effects of exposure to di(2-ethylhexyl)phthalate (DEHP) on maternal body weight during gestation (left panel) and lactation (right panel).

Each point represents the mean.

GH or IGF-I were observed between the control group and the DEHP groups at 9 wk of age (Table 6).

Discussion

In recent years, the issue of endocrine-disrupting chemicals has been the topic of much discussion. Nagel *et al.*[19] and vom Saal *et al.*[20] reported that *in utero* exposure to low doses of bisphenol A (2 and/or 20 μg/kg/day) affects prostate and preputial gland weight and decreases daily sperm production efficiency in mouse offspring; moreover, their results indicated that exposure to low doses of xenoestrogens during a critical period can affect the reproductive organ systems of male offspring. On the other hand, other investigators have failed to find such effects in mouse offspring when using an identical experimental design[21,22]. Thus, the issue of low-dose exposure to these potential endocrine-disrupting chemicals remains a matter of debate among investigators. Hence, as more refined analytical methods become available, risk assessment for previously characterized chemical substances should be repeated.

Table 2. Number of subjects examined

Group	DEHP dose (mg/kg/day)	No. of offspring examined			
		Age (wk)	1	3	9
Control	0	Male	8	8	8
		Female	6	8	8
DEHP	25	Male	10	7	7
		Female	11	7	7
DEHP	100	Male	13	8	7
		Female	9	8	8
DEHP	400	Male	9	7	6
		Female	7	7	7

Embryo-fetotoxicity and teratotoxicity of DEHP have been studied in mice[4-8] and rats[8-10]. These studies were conducted to elucidate whether in utero exposure to high doses of DEHP induces embryotoxicity and/or teratogenicity. The doses used in these previous studies were far in excess of human

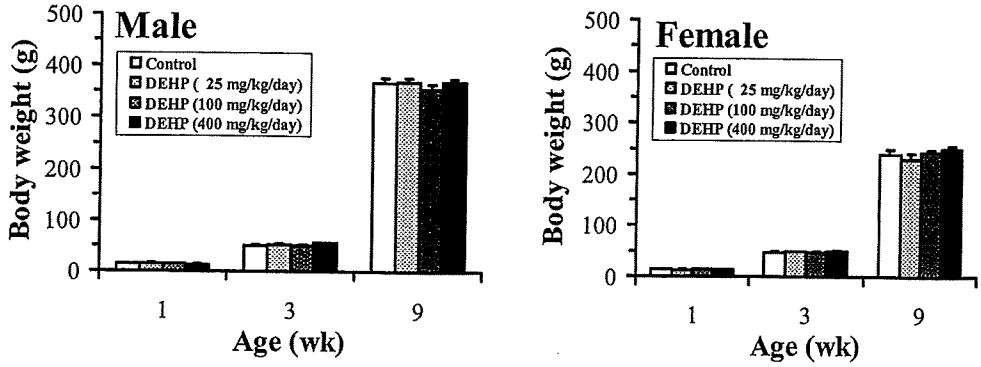

Fig. 2. Effects of maternal exposure to DEHP on postnatal body weight of offspring.
Body weights of male (left panel) and female (right panel) offspring are shown at 1, 3 and 9 wk of age. Each column and vertical bar represent the mean and SEM, respectively. There were no significant differences among groups.

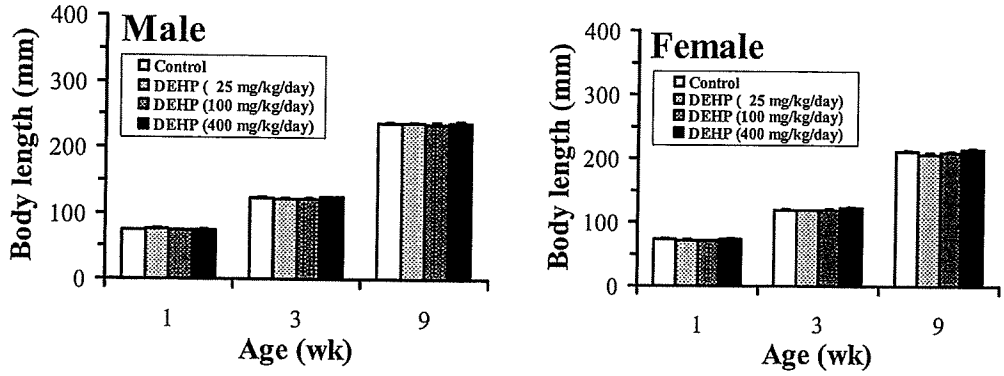

Fig. 3. Effects of maternal exposure to DEHP on postnatal body length of offspring.
Body lengths (nose to anus) of males (left panel) and females (right panel) are shown at 1, 3 and 9 wk of age. Each column and vertical bar represent the mean and SEM, respectively. There were no significant differences among groups.

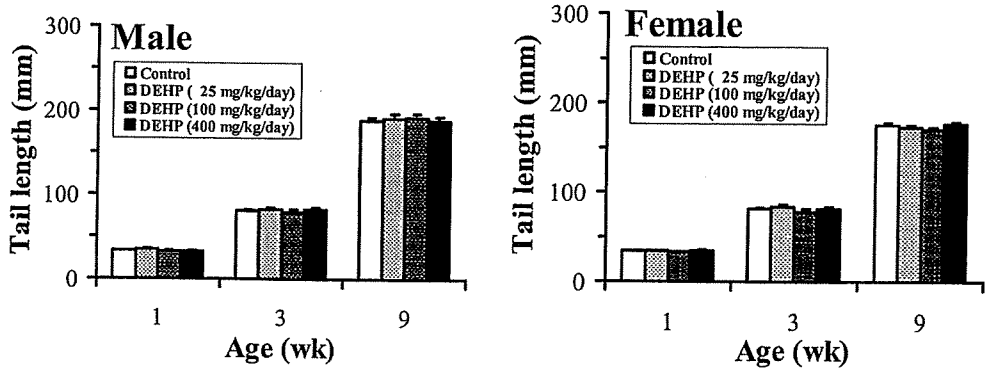

Fig. 4. Effects of maternal exposure to DEHP on postnatal tail length of offspring.
Tail lengths of males (left panel) and females (right panel) are shown at 1, 3 and 9 wk of age. Each column and vertical bar represent the mean and SEM, respectively. There were no significant differences among groups.

Table 3. Organ weights in male offspring

Organ	Group	DEHP dose (mg/kg/day)	Age (wk)		
			1	3	9
Liver (g)	Control	0	0.372 ± 0.011[a]	1.974 ± 0.090	15.55 ± 0.439
	DEHP	25	0.367 ± 0.024	1.984 ± 0.156	16.73 ± 0.560
	DEHP	100	0.334 ± 0.016	1.936 ± 0.138	14.78 ± 0.735
	DEHP	400	0.372 ± 0.037	2.276 ± 0.122	15.83 ± 0.691
Kidneys (g)	Control	0	0.191 ± 0.004	0.618 ± 0.018	2.951 ± 0.093
	DEHP	25	0.188 ± 0.008	0.585 ± 0.037	3.049 ± 0.124
	DEHP	100	0.164 ± 0.007	0.582 ± 0.042	2.842 ± 0.078
	DEHP	400	0.163 ± 0.015	0.632 ± 0.024	3.071 ± 0.092
Testes (g)	Control	0	-[b]	0.222 ± 0.009	3.065 ± 0.095
	DEHP	25	-	0.225 ± 0.014	2.999 ± 0.102
	DEHP	100	-	0.213 ± 0.011	2.834 ± 0.050
	DEHP	400	-	0.241 ± 0.012	3.070 ± 0.092
Prostate (g)	Control	0	-	-	0.443 ± 0.026
	DEHP	25	-	-	0.428 ± 0.033
	DEHP	100	-	-	0.372 ± 0.032
	DEHP	400	-	-	0.358 ± 0.026
Seminal vesicles (g)	Control	0	-	-	1.109 ± 0.057
	DEHP	25	-	-	1.064 ± 0.060
	DEHP	100	-	-	0.979 ± 0.034
	DEHP	400	-	-	1.014 ± 0.096

[a]Values are mean ± SEM. [b] -, not examined.

environmental exposure, and the duration of dosing was limited to the period of gestation. The present study was thus designed to investigate whether in utero and lactational exposure to DEHP affects the development of the next generation. For the main study, we set the highest dose at 400 mg/kg/day to avoid the influence of maternal toxicity and observe the effect of DEHP on the offspring. The exposure period was prolonged to examine the effects of lactational exposure in addition to the effects of gestational exposure. The offspring of dams in which no overt toxicity was observed (0, 25, 100 and 400 mg/kg/day), as determined by body weight and general behavior during gestation and lactation, were used in our study.

In recent years, certain studies have focused on the effects of DEHP and its antiandrogenic action on the hypothalamus-pituitary-gonadal axis[16, 17, 29]; very few studies, however, have reported the effect of DEHP on longer term postnatal development. Hence, it is important to examine the developmental toxicity of DEHP from birth until puberty. In this regard, our study was performed to evaluate the effects of in utero and lactational exposure to DEHP in rat offspring with a special focus on postnatal growth and physical status. We found that somatic and tissue growth and related endocrine landmarks were not affected by DEHP exposure.

Liver weights were slightly increased in the 400 mg/kg/day group for both male and female offspring at 3 wk of age, but no significant differences were observed among treatment groups. DEHP and other phthalates, such as di(2-ethylhexyl) adipate (DEHA) and butylbenzyl phthalate, are peroxisome proliferators that activate peroxisome proliferator–activated receptors and cause liver enlargement[23]. Induction of peroxisome proliferator–activated receptors could result in liver enlargement following DEHP exposure (Table 3, 4). This phenomenon could be an adaptive response following consecutive exposures to DEHP. However, this trend was no longer apparent at 9 wk of age. Since the DEHP groups were not exposed to the compound after 3 wk of age, body burden might be decreased because of metabolic clearance.

In a study of reproductive and accessory organ development following DEHP exposure, dose-dependent reductions in ventral, dorsolateral and/or anterior prostate weight were reported in rat offspring on PND 21 and PND 63 in response to oral administration of DEHP (0, 375, 750 and 1,500 mg/kg/day, GD3–PND21)[16]. This study also showed that DEHP significantly reduced testis weight on PND 21 and PND 63 in a dose-dependent manner. In the present study, on the other hand, testis weights were not

Table 4. Organ weights in female offspring

Organ	Group	DEHP dose (mg/kg/day)	Age (wk)		
			1	3	9
Liver (g)	Control	0	0.338 ± 0.007[a]	1.899 ± 0.117	9.665 ± 0.573
	DEHP	25	0.322 ± 0.015	1.886 ± 0.103	9.279 ± 0.511
	DEHP	100	0.349 ± 0.014	1.808 ± 0.105	9.760 ± 0.505
	DEHP	400	0.367 ± 0.030	2.046 ± 0.092	9.643 ± 0.441
Kidneys (g)	Control	0	0.176 ± 0.006	0.605 ± 0.026	2.039 ± 0.078
	DEHP	25	0.177 ± 0.006	0.593 ± 0.025	1.849 ± 0.091
	DEHP	100	0.179 ± 0.007	0.583 ± 0.023	1.983 ± 0.055
	DEHP	400	0.171 ± 0.007	0.583 ± 0.020	1.959 ± 0.039
Ovaries (mg)	Control	0	-[b]	18.95 ± 0.76	79.57 ± 4.08
	DEHP	25	-	17.80 ± 1.98	74.28 ± 8.14
	DEHP	100	-	14.83 ± 1.83	71.00 ± 4.26
	DEHP	400	-	16.67 ± 0.82	73.42 ± 3.29
Uterus (mg)	Control	0	-	26.03 ± 1.91	327.4 ± 25.3
	DEHP	25	-	30.72 ± 3.95	300.7 ± 14.2
	DEHP	100	-	31.96 ± 2.37	376.3 ± 30.9
	DEHP	400	-	27.82 ± 2.15	340.5 ± 16.1

[a]Values are mean ± SEM. [b] -, not examined.

Table 5. Hormone determinations in male offspring at 9 wk of age

Parameter		DEHP dose (mg/kg/day)			
		0	25	100	400
T_4	(ng/ml)	83.1 ± 6.9[a]	74.1 ± 3.7	73.2 ± 4.7	81.2 ± 7.5
T_3	(ng/ml)	1.74 ± 0.05	1.70 ± 0.06	1.63 ± 0.07	1.81 ± 0.09
GH	(ng/ml)	140.0 ± 35.3	137.3 ± 30.2	130.5 ± 16.3	96.5 ± 19.5
IGF-I	(ng/ml)	669.6 ± 49.0	641.7 ± 57.8	758.6 ± 49.6	743.5 ± 23.8

[a]Values are mean ± SEM.

Table 6. Hormone determinations in female offspring at 9 wk of age

Parameter		DEHP dose (mg/kg/day)			
		0	25	100	400
T_4	(ng/ml)	70.0 ± 7.4[a]	70.7 ± 5.4	67.7 ± 4.8	69.1 ± 6.4
T_3	(ng/ml)	1.88 ± 0.11	1.91 ± 0.06	1.76 ± 0.06	1.79 ± 0.10
GH	(ng/ml)	98.4 ± 9.6	99.5 ± 19.6	121.3 ± 22.4	109.4 ± 19.4
IGF-I	(ng/ml)	499.0 ± 34.4	574.0 ± 34.6	528.6 ± 42.5	632.6 ± 66.0

[a]Values are mean ± SEM.

significantly different between the control and DEHP groups. No significant differences in prostate weights were observed among the groups, although they were reduced in a dose-dependent manner (Table 3). The outcomes of the present study at the highest dose (400 mg/kg/day) were in accordance with those of Moore *et al.*, who conducted a study that used 375 mg/kg/day as the lowest dose[16]. The magnitude of DEHP effects in the present study was much smaller than that found in the study by Moore *et al.*[16]; this discrepancy could be explained by the large difference in dosage range.

Thyroid hormones play pivotal roles in normal growth, neuronal development and metabolism in animals. Endocrine disturbance following chemical exposure is suspected to occur at the embryonic and/or neonatal stage rather than at

the adult stage. An epidemiological study has suggested that toxicants such as polychlorinated biphenyls and dioxins, which are persistent and cumulative compounds in the environment, may affect growth and development through thyroid impairment[24]. Animal studies have reported that 2,3,7,8-tetracholorodibenzo-*p*-dioxin disrupts thyroid homeostasis[25] and causes developmental defects[26] and bone growth deficits[27]. Thyroid hormones are hormonal regulators of bone growth. The principal hormonal regulators during postnatal development are GH and IGF-I, and these hormones, which are regulated by thyroid hormones, are considered biomarkers for longitudinal somatic growth[28]. In the present study, hormonal parameters regarding developmental somatic growth were determined in the offspring to better assess the physical status following DEHP exposure. There were no significant differences in any parameters in male and female rat offspring (Table 5, 6). The fact that normal hormonal parameters were observed in rat offspring following exposure of dams to DEHP (even at high doses) leads us to conclude that postnatal development remains intact in the offspring.

The level of DEHP exposure used in the present study was much greater (~1,000-fold higher) than the estimated intake due to either medical exposure or consumer exposure in adult humans[3]. It was recently suggested that the magnitude of testicular toxicity after DEHP exposure is associated with the duration and/or the route of exposure[14, 29]. Inhalation of DEHP caused an elevation of plasma testosterone without affecting gonadotropin and several steroid enzymes that are involved in testosterone synthesis in male prepubertal rats[14]. These findings suggest that levels of DEHP that cause hormonal disturbance when inhaled may not have the same effect if consumed orally.

In conclusion, our results suggest that prenatal and postnatal exposure to DEHP does not affect postnatal somatic growth or endocrine and physical status of either males or females under the experimental conditions we used. The effects of DEHP exposure, however, remain uncertain and must be clarified using a wider dosage range, an extended exposure period, a side-by-side comparison of different exposure routes and a larger number of animals.

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Original Article

Effects of *in Utero* and Lactational Exposure to Bisphenol A on Thyroid Status in F_1 Rat Offspring

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Abstract: Bisphenol A (BPA), a xenoestrogen, has been reported to mimic the actions of estrogen or to affect the endocrine glands *in vivo* and *in vitro*. In this study, we examined whether *in utero* and lactational exposure to BPA alters thyroid status in rat F_1 offspring. Dams were orally administered various doses of BPA (0, 4 or 40 mg/kg body weight per day) from gestation day (GD) 6 through postnatal day (PND) 20. The BPA and control groups did not differ significantly with respect to plasma thyroxine (T_4) concentration. The thyroid glands from the BPA groups had normal T_4 responses to exogenous thyroid-stimulating hormone *in vivo*. These results suggest that *in utero* and lactational exposure (indirect exposure) to BPA (4–40 mg/kg/day, GD 6 - PND 20) does not affect thyroid functions in the F_1 generation of male and female rats.

Key words: Bisphenol A, Thyroid, Thyroxine, Thyroid-stimulating hormone, Offspring, Rat

Introduction

Bisphenol A (BPA) is very widely used in the manufacture of polycarbonate and epoxy resins, dental sealants and other chemically derived products. BPA released from lacquer coatings has been detected in food cans[1], and it has also been found in saliva collected from subjects treated with dental sealants[2]. Krishnan *et al.* have reported the weak estrogenic action of BPA eluted from a polycarbonate bottle into medium during an autoclaving procedure. They showed that BPA increases the number of progesterone receptors and promotes proliferation of a cultured cell line that originated from a human breast cancer (MCF-7)[3]. It has been estimated that total daily intake is 0.48 μg/kg body weight (BW) per day for 60-kg adults[4].

Reproductive toxicity of BPA has been reported in mice and rats, and low-dose effects of BPA *in vivo* have been observed in mice. BPA increased prostate and preputial gland weight and decreased daily sperm production efficiency in male mice offspring prenatally exposed to BPA at 2 or 20 μg/kg/day from gestation day (GD) 11 through GD 17[5, 6]. Other investigators, however, failed to confirm such effects in mouse offspring using identical experimental designs[7, 8]. Cagen *et al.* reported normal reproductive development in Wistar rat offspring born from mothers supplied with BPA in drinking water at a concentration range of 0.01 to 10 ppm (equivalent to approximately 0.001–4.022 mg/kg/day) for 10 weeks, from the premating day (at 9 weeks old) to the weaning day[9]. In addition, multigeneration reproductive toxicity studies of BPA have been conducted in rats[10, 11]. Both of these studies confirmed that there were no BPA-related effects on developmental and reproductive parameters at low doses of exposure.

Although the reported effects of BPA are not entirely consistent, it has received a great deal of attention with regard to its possible effects on reproductive glands and accessory genital glands, which are due primarily to its estrogenic activity. It has been known that BPA exerts weak estrogenic activity *in vivo*[12] and *in vitro*[13]. BPA may also disrupt thyroid homeostasis because of its structural similarity to thyroid

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Table 1. Treatment design and number of subjects examined

Measurement	Group	Dose (mg/kg/day)	No. of offspring tested			
			Age (wks)	1	3	9
Plasma T_4	Control	0	Male	6	5	5
			Female	8	5	5
	BPA	4	Male	9	6	3
			Female	8	6	5
	BPA	40	Male	1	5	5
			Female	9	5	5
TSH stimulation test	Control	0	Male	–[a]	–	4
			Female	–	–	7
	BPA	4	Male	–	–	2
			Female	–	–	4
	BPA	40	Male	–	–	4
			Female	–	–	4

[a]Not examined.

hormone. *In vitro* studies have demonstrated that BPA binds weakly to the thyroid hormone receptor and suppresses transcriptional activity that is stimulated by tri-iodothyronine (T_3)[14]. On the other hand, BPA does not inhibit the binding of T_3 to the thyroid hormone receptor and does not inhibit the hormonal activity of T_3 to induce growth and growth hormone (GH) production in the rat pituitary cell line GH3[15]. Although BPA is suspected to mimic thyroid hormone by modulating the thyroid hormone receptor, the *in vitro* effect of BPA on thyroid function/action is controversial, and the *in vivo* effect of BPA on thyroid status is unknown.

In the present study, we administered relatively high oral doses of BPA to pregnant rats and measured the effects on thyroid parameters including plasma thyroxine (T_4) levels at 1, 3 and 9 weeks of age in male and female offspring. To better assess thyroid function, we also measured *in vivo* T_4 responsiveness of thyroid glands to exogenous thyroid-stimulating hormone (TSH) at 9 weeks of age.

Materials and Methods

Animals

BPA (purity >99.8%, Cat#: 280-08561, Lot#: HCE9312) and corn oil (Cat#: 034-17015) were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. A total of 24 pregnant female rats (Crj: CD (SD) IGS strain, 9 weeks of age) at GD 3 were purchased from Charles River Japan, Inc. (Tsukuba) and housed separately and maintained under controlled temperature (23 ± 1°C), humidity (55 ± 5%) and a 12-h light-dark cycle (08:00–20:00) conditions throughout the study. The presence of a copulatory plug defined GD 0. A standard laboratory diet (CE-2, Clea Japan, Inc., Tokyo, Japan) and drinking water were available *ad libitum*. Dams were randomly divided into four groups (6 pregnant rats per group) and weighed once a day from GD 3 through PND 20 (except for GD 4–5). The BPA-exposed groups were dosed by oral gavage with 4, 40 or 400 mg/kg BW per day of BPA in corn oil vehicle (10 ml/kg BW) once daily between 08:30 and 09:30 from GD 6 through PND 20, and the control group was given the same amount of corn oil during the same period. The litter size was standardized to ten (male:female = 5:5, if possible) between 10:00 and 11:00 on PND 7 (1 week of age). Examinations were performed as soon as possible after offspring were culled. On PND 21, the remaining offspring were weaned, and thereafter males and females were housed separately per litter. A pair of male and female offspring from each dam was dissected at 3 and 9 weeks of age. The remaining offspring were used for brain function and behavioral effects (not reported here). Table 1 shows the treatment design and number of subjects examined. The 400 mg/kg/day group was excluded from further analysis because of its excessive maternal toxicity[16].

Hormone assay

Plasma samples were prepared as described previously[16]. Plasma T_4 levels were determined by a chemiluminescence immunoassay (ACS; Centaur; Chiron Corp., Emeryville, CA). The plasma T_4 levels were also used as basal values for the TSH stimulation test (see below).

TSH stimulation test

The BPA-exposed and control groups of both sexes at 9