Korean J Biol Sci 5: 327-331, 2001

Effects of Polychlorinated Biphenyls on the Expression of KAP3 Gene Involved in the 'Critical Period' of Rat Brain Sexual Differentiation

Chae Kwan Lee[2], Han Seung Kang, Bu Il June, Byung Ju Lee[1], Deog Hwan Moon[2], and Sung Goo Kang*

Department of Biology, College of Bioengineering, Inje University, Kimhae 621-749, Korea;
[1]Department of Biology, College of Natural Sciences, Ulsan University, Ulsan 680-749, Korea;
[2]Institute of Industrial Medicine, Inje University, Kimhae 621-749, Korea

Key Words:
Brain sexual differentiation
Polychlorinated biphenyls
KAP3

There is a critical developmental period during which brain sexual differentiation proceeds irreversibly under the influence of gonadal hormone. Recently, kinesin superfamily-associated protein 3 (KAP3) gene expressed during the 'critical period' of rat brain differentiation was identified by us (Choi and Lee, 1999). KAP3 functions as a microtubule-based motor that transports membranous organelles anterogradely in cells, including neurons (Yamazaki et al., 1996). mRNA level of KAP3 gene markedly increased before the initiation of puberty. Neonatal treatment of estrogen clearly inhibited the prepubertal increase in KAP3 mRNA level (Choi and Lee, 1999). In the present study, we aimed to investigate the effects of polychlorinated biphenyls (PCBs), as endocrine disruptors (EDs) on the expression of KAP3 gene during the 'critical period' of rat brain development. In our data, PCBs significantly decreased the expression of KAP3 gene in the fetal (day 17) and the neonatal (day 6 after birth in) male and female rat brains. The body weight and the breeding ability were significantly decreased in the PCBs-exposed rats compared with the control. These results showed that PCBs affect the transcriptional level of brain sexual differentiation related gene, KAP3, in the fetal and the neonatal rat brains. The maternal exposure to the PCBs may lead to toxic response in embryonic brain sexual differentiation and breeding ability after sexual maturation. This study indicates that KAP3 gene may be useful as a gene marker to analyze the molecular mechanism of toxic response in the animal brain development and sexual maturation exposed to PCBs.

A large number of man-made chemicals released into the environment have the potential to disrupt the endocrine system of animals and humans. They mimic the effect of natural hormones or neurotransmitters by recognizing their binding sites, or they antagonize the effects of endocrinous hormones or neurotransmitters by blocking their interaction with their physiological binding sites. Interaction of the environmental endocrine disruptors with animals or humans during ontogeny may have deleterious effects on differentiation of reproductive structures and functions (Dohler., 1998). Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that accumulate in the food chain (Gallant et al., 2000). They act as estrogenic chemicals (Kester et al., 2000). Exposure to the estrogenic chemicals during the 'critical periods' in fetal life can alter the development of reproductive organs, the neuroendocrine system, and subsequent behavior (Palanza et al., 1999). PCBs are known to elicit a spectrum of toxic responses in humans and laboratory animals, such as reproductive and developmental toxicity, body weight loss, immunosuppressive effects, hepatotoxicity, thyroid atrophy and disruption of the homeostasis of steroid hormones (Ness et al., 1993; Mousa et al., 1996; Rice and Hayward 1999; Fielden et al., 2001). Embryonic abnormality and low fertility occurred after the exposure of germ cells to PCBs (Mousa et al., 1996).

Sex-specific behaviors and physiological mechanisms have evolved to facilitate reproduction and are determined by the central action of steroid hormones during brain development (Simerly et al., 1997). During the 'critical period' of brain sexual differentiation, estrogen exerts organizational effects on differentiation of the

* To whom correspondence should be addressed.
Tel: 82-055-320-3213, Fax: 82-055-336-7706
E-mail: biosgkan@ijnc.inje.ac.kr

sexually dimorphic brain area. Neonatal treatment of estrogen permanently changed sex-related brain activities and behaviors and led to masculinization in adult mammals (Parsons et al., 1980; Toran-Aller, 1984; McEwen et al., 1987). Recently, KAP3 gene was cloned from mouse and rat brain by our group and others (Hirokawa, 1997; Choi and Lee, 1999). Two isoforms, KAP3A and KAP3B, are derived from a single gene by alternative splicing. KAP3 is a globular protein and binds to the tail region of the kinesin superfamily protein (KIF) 3A/KIF3B heterodimer and may regulate membrane binding of the KIF3A/KIF3B complex (Yamazaki et al., 1996). KIF functions as a microtubule-based motor that transports membranous organelles anterogradely in cells, including neurons (Yamazaki et al., 1995). KAP3 mRNA significantly reached the peak level at 28-day of age and decreased thereafter in both the female and male rat hypothalamus. On the contrary, the neonatal treatment of estrogen removed the 28-day peak (Choi and Lee, 1999). In this paper, we aimed to investigate the effects of PCBs on the expression of KAP3 gene during the 'critical period' of rat brain development and on the breeding ability.

Materials and Methods

Animals and tissue preparation

Pregnancy of Sprague Dawley rats were checked with the presence of a copulatory plug or sperms in the vaginal smear, which was defined as pregnant day 0. PCBs (Aroclor 1254, Fisher Scientific) were mixed with sesame oil (Sigma). For prenatal injection, pregnant rats were intraperitoneally injected with PCBs (25 mg/kg BW) on day 16 of gestation. Rats were sacrificed on gestational day 17 and the fetal brains were removed. For neonatal injection, rats were intraperitoneally injected with PCBs (25 mg/kg BW) 5 d after birth. On day 6 after birth, rats were sacrificed and the brains were collected. Control animals were injected with sesame oil containing the same amount of ethanol.

Body weights and breeding ability

PCBs (25 mg/kg) were intraperitoneally injected into pregnant (day 16) (n = 4) or neonatal (day 1-5 day) (n = 30) rats. Changes of body weights were measured on day 6 after birth. The rats were divided into four groups (normal male and female, normal male and exposed female, exposed male and normal female, and exposed male and exposed female) at seven weeks after birth and mated for two weeks to survey the breeding ability of the rats exposed to PCBs. Twenty couples were mated per each group and the breeding ability was defined by the pregnancy rate and survival indices pregnancy.

RNA extraction and Northern blot analysis

Total RNA was extracted using the Tri-reagent (Sigma). For Northern blot analysis, RNA samples (30 μg) were fractionated on a 1% agarose/2.2 M formaldehyde gel at 100 V for 1.5 h, transferred to Nytran membranes (0.45 μm pore size; Schleicher and Schuell). The membranes were prehybridized with 10 mL of hybridization buffer for 2 h. The hybridization buffer consisted of 50% deionized formamide, 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate), 5×Denhardt's solution (1× Denhardt's solution = 0.01% polyvinylpyrrolidone, 0.01% Ficoll, and 0.01% BSA), 0.1% SDS and 2 mg of heat-denaturated salmon sperm DNA. Hybridization was carried out in a hybridization incubator (Stuart Scientific) with hybridization buffer plus ^{32}P-dCTP labeled KAP3 cDNA probe at 42℃ for 15 h. Membranes were washed at high stringency and exposed to X-ray film (X-OMAT AR, Kodak) for 1-4 d.

cDNA probe

Cloned KAP3 cDNA fragments were labeled for cDNA probes with ^{32}P-dCTP using the oligolabeling kit (Pharmacia Co.). Free ^{32}P-dCTP was removed using the Nick column (Pharmacia). The specific activity of probe was about 1×10^9 cpm/mL.

Statistics

Statistical comparisons between the groups were analyzed by either unpaired student's t-test for two groups or one-way analysis for variance for more than two groups. The level of statistical significance was set at $p < 0.05$.

Results

Changes of bodyweight in rats exposed to PCBs

To investigate the changes of body weight in rats exposed to PCBs during prenatal or neonatal ages, PCBs was intraperitoneally injected into the pregnant (day 16) and the neonatal (day 1-5 d) rats. The changes of body weights were measured on day 6 after birth. Body weights were decreased in the rats exposed to PCBs in both prenatal and neonatal groups (Fig. 1). Maternal injection of PCBs decreased the bodyweights by about 18%. Neonatal injection of PCBs decreased the bodyweights by about 26% compared with the control group.

Effects of PCBs on the KAP3 mRNA levels in prenatal and neonatal rat brain

Pregnant rats were intraperitoneally injected with PCBs on pregnant day 16. Neonatal rats were also injected with PCBs for 5 d after birth. Rats were sacrificed at day 17 of gestation or at day 6 after birth. KAP3 mRNA levels in the prenatal and neonatal rat brains were

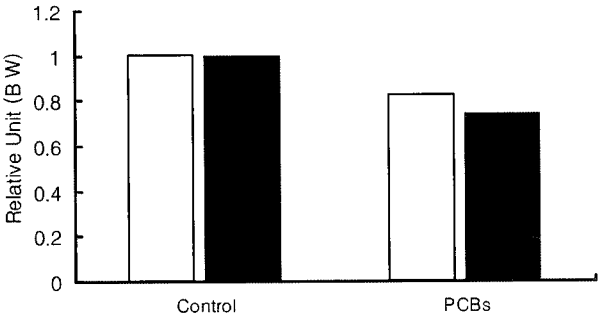

Fig. 1. Changes in the bodyweight of new born rats after the injection with PCBs during prenatal and neonatal age. Prenatal (□, n = 30) or neonatal (■, n=30) rats were injected with PCBs (25 mg/kg BW)/ sesame oil or sesame oil (control) at gestational day 16 or for 5 d after birth. Bodyweights were measured at day 6 after birth and expressed as relative units over control value of 1.0.

analyzed by Northern blot hybridization. KAP3 mRNA in the rat brain showed a single transcript of about 3.5 kb (Fig. 2A) as previously reported (Yamazaki et al., 1996). In the PCBs-treated prenatal rat brains, the level of KAP3 mRNA was decreased by about 38% ($p < 0.05$) in the male and 13% in the female brains compared with the control (Fig. 2B). In the PCBs-treated neonatal rat brains, the level of KAP3 mRNA was decreased by about 15% in the male and 24% in the female brains ($p < 0.05$) compared with the control (Fig. 3).

Breeding ability

To survey the breeding ability of rats exposed to PCBs

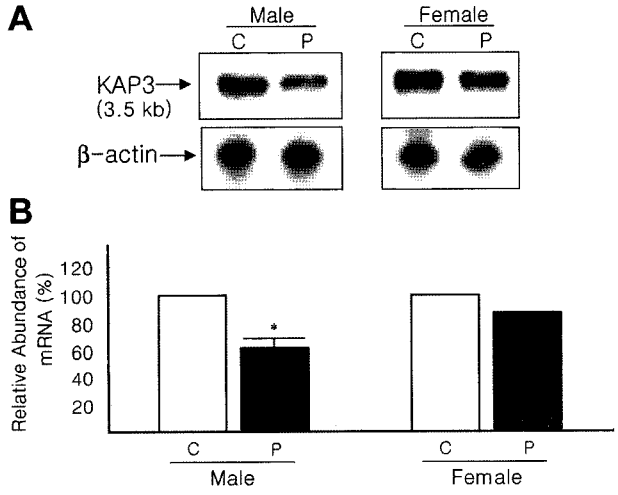

Fig. 2. Northern blot analysis of KAP3 mRNA in the male and the female fetus brain obtained from pregnant rats exposed to PCBs. Pregnant rats were exposed to the PCBs (25 mg/kg BW) at gestational day 16, sacrificed at day 17 and fetal brains were removed. **A.** Replicate northern blots containing 30 μg of total RNAs were cRNA probed to analyze the mRNA levels of KAP3 gene and were exposed to X-ray film for 48 h. **B.** KAP3 mRNA levels were normalized with β-actin mRNA levels and relative values were calculated as % of control value. Each point represents the mean of KAP3 mRNA levels from three times experiments (*, $p < 0.05$). C: control, P: PCBs.

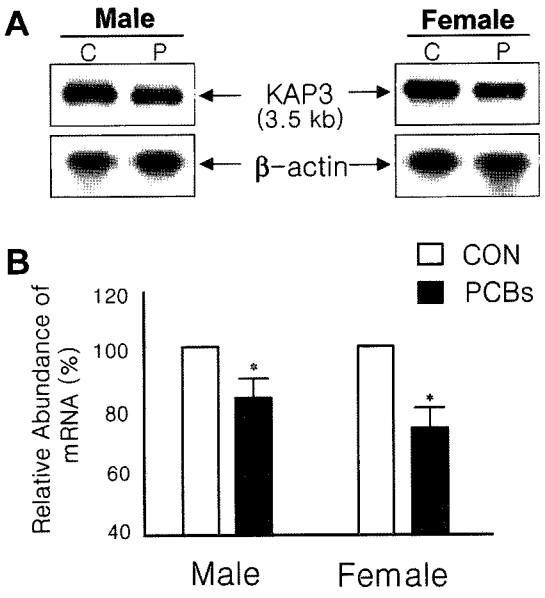

Fig. 3. Northern blot analysis of KAP3 mRNA in the neonatal male and female rat brain exposed to PCBs. Neonatal rats were injected with PCBs (25 mg/kg BW) for 5 d after birth. Rats were sacrificed at day 6 and brains were removed. **A.** Replicate northern blots containing 30 μg of total RNAs were cRNA probed to analyze the mRNA levels of KAP3 gene and were exposed to film for 48 h. **B.** Relative levels of KAP3 mRNA were normalized with β-actin levels and calculated as % of control values. Each point represents the mean of KAP3 mRNA levels obtained from three experiments (*$p < 0.05$). C: control, P: PCBs.

during the prenatal and neonatal ages, the rats were divided into four groups at seven weeks after birth and mated (n = 20) for two weeks. The breeding ability was defined by the pregnancy rate and survival indices pregnancy. The pregnancy rates of PCBs exposed groups were clearly decreased compared to the control

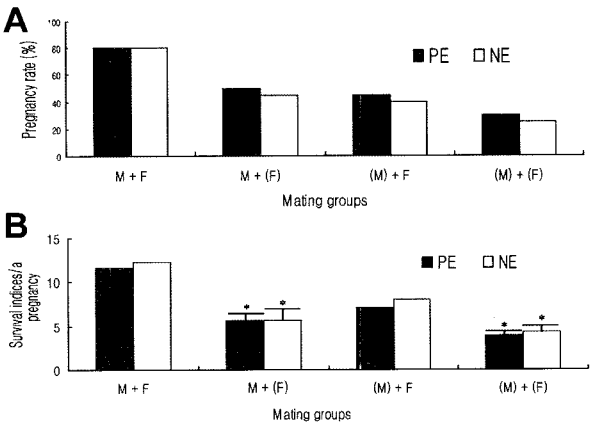

Fig. 4. Pregnancy rates and survival indices pregnancy of rats exposed to PCBs during the prenatal or the neonatal age. The rats were divided into four groups at 7 weeks after birth and mated (n = 20) for 2 weeks. To survey the pregnancy rates (A) and survival indices/a pregnancy (B) of rats exposed to PCBs during the prenatal or neonatal age, data were presented as means ± SEM, followed by the number of survival indices/a pregnancy. *, Statistically significant difference from control ($p < 0.05$). PE: prenatal exposed, NE: neonatal exposed, ( ): Male or female rats exposed to PCBs.

group (Fig. 4A). The pregnancy rates of normal male and female groups were 80%. In the prenatal PCBs exposed groups, the pregnancy rates of normal male-PCBs exposed female and PCBs exposed male-normal female groups were 50% and 45%, respectively. PCBs exposed male-PCBs exposed female group was 30%. In the neonatal PCBs exposed groups, the pregnancy rates of normal male-PCBs exposed female and PCBs exposed male-normal female groups were 45% and 40%, respectively. PCBs exposed male-PCBs exposed female group showed 25%. The survival indices of offspring pregnancy were also significantly decreased in the PCBs exposed groups compared to the control group (Fig. 4B). In the prenatal PCBs exposed groups, survival indices pregnancy of normal male-normal female group was 11.56 ± 3.12. Normal male-PCBs exposed female, PCBs exposed male-normal female, and PCBs exposed male-PCBs exposed female groups showed 5.60 ± 1.28, 7.00 ± 2.25, and 3.83 ± 0.57 respectively. In the neonatal PCBs exposed groups, survival indices pregnancy of normal male-normal female group was 12.25 ± 3.45. Normal male-PCBs exposed female, PCBs exposed male-normal female, and PCBs exposed male-PCBs exposed female groups resulted in 5.67 ± 0.97, 8.02 ± 2.83, and 4.20 ± 0.63, respectively.

Discussion

In the present study, we showed the effects of PCBs on body weights, breeding abilities and expression of KAP3 gene as related to the brain sexual differentiation. PCBs exposure induced bodyweight loss. This effect of PCBs on bodyweight agree well with the previous report that PCBs elicit toxic responses in human and laboratory animals including body weight loss, immunosuppressive effects, hepatotoxicity and other factors (Ness et al., 1993; Mousa et al., 1996; Rice and Hayward 1999; Fielden et al., 2001). Fig. 1 showed that maternal or neonatal injection of PCBs decreased the bodyweights by 18% to 26% compared with the control.

In the rodent, the 'critical period' of brain sexual differentiation is usually from late pregnancy to 7 to 10 days after birth (von Saal and Bronson, 1980; Weisz and Ward, 1980). During the 'critical period', estrogen exerts organization effects on the differentiation of a number of sexually dimorphic brain areas. Neonatal injection of estrogen permanently modulates sex-related brain activation and behaviors leading to masculinization in adult mammals (Parsons et al., 1980; Toran-Aller, 1984; McEwen et al., 1987). However, there are few reports that have studied regulation of gene expression related to brain sexual differentiation during prenatal or neonatal age. In the previous study, we applied PCR differential display using RNA samples derived from estrogen sterilized rat (ESR) hypothalamus. About 100 out of more than 1000 RNA species examined displayed differential expression patterns between a 60-day old control rat and ESR. Sequence analysis of differentially amplified PCR products showed homology with mouse KAP3 and several cDNAs previously described by others. We have introduced antisense KAP3 oligodeoxynuclotide (ODN) into the lateral ventricle of immature female rat to suppress the KAP3 mRNA level (Choi and Lee, 1999). The administration of the ODN decreased KAP3 mRNA level and resulted in a significant delay of puberty, as described above (Hayashi and Aihara, 1989; Faber and Hughes, 1991; Faber et al., 1993; Pinilla et al., 1993). Neonatal treatment of estrogen attenuated the 28-day peak of KAP3 mRNA level (Choi and Lee, 1999). These data suggest that KAP3 gene is involved in the brain sexual differentiation and that its expression is suppressed by estrogenic effect. Thus we examined the effects of PCBs on the mRNA levels of KAP3 in prenatal and neonatal rat brains. The exposure to estrogenic chemicals during the 'critical periods' in fetal life can alter the development of reproductive organs, neuroendocrine system, and subsequent behavior (Palanza et al., 1999). PCBs are ubiquitous environmental contaminants that bioaccumulate in the food chain and exhibit estrogen-like activity in humans and other animals (Gallant et al., 2000). Our Northern blot results showed a single transcript of about 3.5 kb (Fig. 2A) as previously reported (Tamazaki et al., 1996). Fig. 2B clearly demonstrated that KAP3 mRNA levels were decreased in the PCBs-treated prenatal male and female rat brains. In the PCBs-treated neonatal male and female rat brains, the similar result was obtained (Fig. 3). These results implied that PCBs may disturb the brain sexual differentiation mechanisms via down regulation of KAP3 gene expression. KAP3 is a KIF3A/3B associated protein. Many KIFs have been cloned and analyzed as a microtubule-based motor that transports membranous organelles anterogradely in cells, including neurons (Brady, 1985; Vale et al., 1985; Hirokawa et al., 1991; Hirokawa, 1993; 1997). Biological function of KAP3 in brain sexual differentiation is not clear yet. But it may be involved in mammalian brain development. Moreover, PCBs decreased the pregnancy rates (Fig. 4A) and survival indices of offspring (Fig. 4B) and induced morphological abnormality on the development of genitals (data not shown). Taken together, these results suggested that exposure to known estrogenic EDs such as PCBs during the 'critical period' of rat brain sexual differentiation leads to disruption of the transcriptional regulation of KAP3 gene which may be related to the abnormalities in brain sexual differentiation and genital morphology. KAP3 gene may be useful as a gene marker to analyze the molecular mechanism of toxic response in EDs exposed animal nerve tissues.

Acknowledgements

This work was supported by grant from Genetic Engineering Program (1998) of Korea Ministry of Education and KOSEF through the Biohealth Products Research Center at Inje University (2001).

Reference

Barraclough CA (1961) Production of anovulatory, sterile rats by single injections of testosterone propionate. *Endocrinology* 68: 62-67.

Barraclough CA (1979) Sexual differentiation of cyclic gonadotropin secretion. *Adv Biosci* 25: 433-450.

Brady ST (1985) A novel brain ATPase with properties expected for the fast axonal transport motor. *Nature* 317: 73-75.

Choi EJ and Lee BJ (1999) Identification of genes involved in the onset of female puberty of rat. *Korean J Biol Sci* 3: 319-329.

Dohler KD (1998) Influence of hormones and hormone antagonists on sexual differentiation of the brain. *Arch Toxicol Suppl* 20: 131-141.

Faber KA, Ayyash L, and Dixon S (1993) Effect of neonatal diethylstilbestrol exposure on volume of the sexually dimorphic nucleus of the preoptic area of the hypothalamus and pituitary responsiveness to gonadotroin-releasing hormone in female rats of known anogenital distance at birth. *Biol Reprod* 48: 947-951.

Faber KA and Hughes CL (1991) The effect of neonatal exposure to DES, genistein, and zearalenone on pituitary responsiveness and SDN-POA volume in the castrated adult rat. *Biol Reprod* 45: 649-653.

Fielden MR, Halgren RG, Tashiro CH, Yeo BR, Chittim B, Chou K, and Zacharewski TR (2001) Effects of gestational and lactational exposure to Aroclor 1242 on sperm quality and in vitro fertility in early adult and middle-aged mice. *Reprod Toxicol* 15: 281-292.

Gallant TL, Singh A, and Chu I (2000) PCB 118 induces ultra structural alterations in the rat liver. *Toxicology* 145: 127-134.

Gerall AA, Hendricks SE and Johnson LL (1967) Effects of early castration in male rats adult sexual behavior. *J Comp Physiol.* 64: 206-212.

Gorski RA (1963) Modification of ovulatory mechanisms by postnatal administration of estrogen to the rat. *Am J Physiol* 205: 842-844.

Gorski RA (1985) Sexual dimorphisms of the brain. *J Anim Sci* 61: 38-61.

Greenough WT, Carter CS, and Steerman C (1977) Sex differences in dentritic patterns in hamster preoptic area. *Brain Res* 126: 63-72.

Hayashi S and Aihara M (1989) Neonatal estrogenization of the female rat: a useful model for analysis of hypothalamic involvement of gonadotropin release. *Zool Sci* 6: 1059-1068.

Hayashi S, Aihara M, and Wakabayashi K (1991) Content and distribution pattern of luteinizing hormone-releasing hormone (LHRH) in the hypothalamus of neonatally estrogenized female rats. *Neurosci Res* 12: 366-378

Hirokawa N, Sato-Yoshitake R, and Kobayashi N (1991) Kinesin associates with anterogradely transported membranous organelles *in vivo*. *J Cell Biol* 114: 295-302.

Hirokawa N (1993) Axonal transports and the cytoskeleton. *Curr Opin Neurobiol* 3: 724-731.

Hirokawa N (1997) The mechanisms of fast and slow transport in neurons: identification and characterization of the new kinesin superfamily motors. *Curr Opin Neurobiol* 7: 605-614.

Kester MH, Bulduj S, and Tibboel D (2000) Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* 141: 1897-1900.

Matsumoto A and Arai Y (1981) Neuronal plasticity in the differentiated hypothalamic arcuate nucleus of adult female rats and its enhancement by treatment with estrogen. *J Comp Neurol* 197: 197-205.

McEwen BS, Jones KJ, and Pfaff DW (1987) Hormonal control of sexual behavior in the female rat: molecular, cellular and neurochemical studies. *Biol Reprod* 36: 37-45.

Mousa MA, Quensen JF, and Chou K (1966) Microbial dechlorination alleviates inhibitory effects of PCBs on mouse gamete fertilization *in vitro*. *Environ Sci Technol* 30: 2087-2092.

Ness DK, Schantz SL, and Moshtaghian J (1993) Effects of prenatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. *Toxicol Lett* 68: 311-323.

Palanza P, Parmigiani S, and Liu H (1999) Prenatal exposure to low doses of the estrogenic chemicals diethylstillbestrol and o,p-DDT alters aggressive behavior of male and female house mice. *Pharmacol Biochem Behav* 64: 665-672.

Parsons B, MacLusky NJ, and Krey L (1980) The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior. *Endocrinology* 107: 774-779.

Pinilla L, Trimino E, and Garnelo P (1993) Changes in pituitary secretion during the early postnatal period and anovulatory syndrome induced by neonatal oestrogen or androgen in rats. *J Reprod Fertil* 97: 13-20.

Raisman G and Field PM (1973) Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. *Brain Res* 54: 1-29.

Rice DC and Hayward S (1999) Effects of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on behavior (concurrent random interval-random interval and progressive ratio performance) in rats. *Neurotoxicol Teratol* 21: 679-687.

Simerly RB, Zee MC, and Phedleton JW (1997) Estrogen receptor-dependent sexual differentiation of dopaminergic neurons in the preoptic region of the mouse. *Proc Natl Acad Sci USA* 94: 14077-14082.

Toran-Aller CD (1984) Gonadal hormones and brain development: implications for the genesis of sexual differentiation. *Ann NY Acad Sci* 435: 101-111.

Vale RD, Reese TS, and Sheetz MP (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 42: 39-50.

vom Saal FS and Bronson FH (1980) Sexual characteristics of adult female mice are correlated with aystheir blood testosterone levels during prenatal development. *Science* 208: 597-599.

Weisz J and Ward IL (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology* 106: 306-316.

Yamazaki H, Nakada T, and Okada Y (1995) KIF3A/B: A heterodimeric kinesin superfamily protein that works as a microtuble plus end-directed motor for membrane organelle transport. *J Cell Biol* 130: 1387-1399.

Yamazaki H, Nakata T, and Okada Y (1996) Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. *Proc Natl Acad Sci USA* 93: 8443-8448.

[Received October 8, 2001; accepted November 2, 2001]