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The Effect of Polybrominated Biphenyl (PBB) on Testes, Adrenal, and Pituitary Function in the Rat[1] (41355)

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Abstract. In an earlier study we reported a dose- and time-dependent effect of polybrominated biphenyls (PBB) on thyroid function in the rat. In the present study we report the effects of PBB administration to male rats on testis, adrenal, and pituitary endocrine function. PBB were administered by gavage at 1, 3, or 6 mg/kg/day for 20 days. There was no effect of PBB administration on adrenal or testis weights, despite a significant increase in liver weight at all dosages, nor were plasma corticosterone or testosterone levels altered by treatment. At the highest PBB dosage used, there was a significant reduction in plasma prolactin levels. After 20 days of PBB administration at 3 mg/kg/day, tissue concentration of PBB in the testes was only 8.7 ± 3.7 μg/g, while concentration in the adrenal was 93.7 ± 15.3 μg/g, much greater than levels reported earlier for thyroid and liver. After cessation of PBB and 5 months of dietary restriction treatment, adrenal PBB concentration significantly increased to 481.1 ± 105.9 μg/g, suggesting a mobilization from adipose tissue and a preferential sequestration in the adrenal glands.

Numerous studies have demonstrated the general toxicity of polybrominated biphenyls (PBB), with detrimental effects reported on growth and reproduction in several species (1–4). A significant omission from these studies is in the area of endocrine physiology. A recent report from this laboratory (5) demonstrated that PBB administration had a pronounced effect on thyroid function in rats as exemplified by a dose- and time-dependent decrease in serum thyroxine associated with a corresponding increase in TSH. Moreover, this effect of PBB on thyroid function persisted as long as 5 months following the cessation of PBB administration. In the present study, we report on the effect of PBB administration on testis, adrenal, and pituitary function as reflected by changes in plasma testosterone, corticosterone, and prolactin and on both testis and adrenal weights.

Materials and Methods. Adult male Sprague–Dawley rats weighing 300–500 g at the start of the experiment were housed individually in a quarantine biohazard laboratory and maintained on a standard 12-hr dark/12-hr light schedule with Purina Rat Chow and water available *ad libitum*. Experiments consisted of comparable numbers of animals receiving 1, 3, or 6 mg/kg PBB per day (obtained from the National Institute of Environmental Health Science, Lot No. FF-1312-FT,-3) incorporated into lecithin liposomes (6). Control rats received gavages of lecithin liposomes alone. PBB was administered Monday through Friday between 0830 and 1000 hr for 20 treatment days and administration was completed in 26 days. Following the last PBB or control gavage, animals were decapitated, alternating experimental with control, and blood was collected in heparinized tubes. Plasma was separated and stored at −20° until analyzed for testosterone, corticosterone, and prolactin by radioimmunoassay. Body

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weights were recorded, as well as liver, adrenal, and testis weights. Organs were stored frozen for subsequent analysis of PBB content.

AM and PM blood samples for plasma corticosterone analysis were obtained from the 3 mg/kg treatment and the control group 2 days prior to sacrifice. The PM (1700 hr) blood samples were obtained from the tail vein within 2 min after removal from the home cage and the AM (0800) samples obtained 15 hr later.

A single group of rats treated with 3 mg PBB/kg was placed on a restricted food intake to maintain their body weights at 80% of that measured at the end of the PBB treatment period in order to use these animals in operant conditioning studies (6). Adrenals from these animals were analyzed for PBB content 5 months after treatment and we have presented the thyroid PBB concentration and plasma thyroxine levels of these animals in an earlier report (5).

Radioimmunoassay. Prolactin. Reagents for the radioimmunoassay of rat prolactin were supplied by the Rat Pituitary Hormone Distribution Program NIAMD). The lower limit of detectability was 15 ng/ml. All samples were assayed in a single assay. The intraassay coefficient of variation was 15%.

Testosterone. A highly specific antiserum, generously provided by Dr. P. N. Rao, was used for the RIA of testosterone, and has previously been validated (7). Assays were performed on ether extracts of plasma without chromatographic separation. Intra- and interassay coefficients of variation are 5.8 and 13.5%, respectively.

Corticosterone. Plasma corticosterone was measured by radioimmunoassay following chloroform:dichloromethane (2:1) extraction. The antiserum was generously provided by Dr. Walter Morishige (University of Hawaii). The intra- and interassay coefficients of variation were 6.5 and 11.8%, respectively. The antisera had no significant crossreaction with any major circulating steroids of the rat (Morishige, personal communication).

PBB analyses were done by gas–liquid chromatography with electron capture detection as previously described (5).

Statistical analyses were done using Student's t test.

Results. *Effect of PBB administration on organ weights.* As noted in our earlier report (5), PBB treatment had no significant effect on body weight, when compared to gavage controls. A significant increase in liver weight following PBB administration was observed at all three doses; however, there was no significant effect at any PBB dosage on either the mean adrenal or testes weights (Table I).

Effect of PBB administration on plasma endocrine parameters. No significant alterations at any PBB dosage were observed on either plasma testosterone (Table II) or plasma corticosterone (Table II). In the 3 mg/kg treatment group, the normal AM–PM difference in basal corticosterone levels persisted despite PBB treatment (Table III). A significant reduction in plasma prolactin level was seen at the 6 mg/kg dose compared to vehicle treated controls (Table II).

Effect of PBB administration on adrenal and testes PBB concentrations. PBB were not detectable in adrenals or testes from control animals. PBB (3 mg/kg/day) administration for 20 days resulted in mean ($\pm$ SE; $n = 7$) adrenal tissue concentration of 93.7 $\pm$ 15.3 μg/g, whereas testis levels of PBB were only 8.7 $\pm$ 3.7 μg/g ($n = 8$). Following a 5-month post-treatment period in which food was restricted, adrenal concentration of PBB was significantly increased ($P < 0.0025$) to 481.1 $\pm$ 105.9 μg/g ($n = 7$) (Fig. 1).

Discussion. We have reported a time- and dose-dependent reduction in plasma thyroxine following PBB administration (5). The present study examines the effect of 1, 3, or 6 mg/kg PBB on the physiology of other endocrine organs. At these dose levels, no significant alteration of either testicular or adrenal weight occurred, despite significant increases in liver weight. Plasma testosterone was not affected by PBB administration. This is in agreement with Sleight *et al.* (8), who did not detect histological lesions in the testes of PBB-treated rats. Indirect evidence of a PBB effect on testis function has been reported by Jackson and Halbert (9), who noted tes-

TABLE I. EFFECT OF 20 DAYS OF PBB ADMINISTRATION ON BODY, LIVER, TESTES, AND ADRENAL WEIGHTS IN MALE RATS

Treatment	Body wt (g)	Liver wt[a] (g)	Testes wt (g)	Adrenal wt (mg)
Control (n = 8)	315 ± 10[b]	12.3 ± 0.6	3.8 ± 0.1	63.0 ± 3.8
PBB, 1 mg/kg (n = 8)	308 ± 10	16.5 ± 0.3*	3.7 ± 0.1	70.3 ± 3.7
Control (n = 8)	433 ± 11	14.5 ± 1.1	3.3 ± 0.1	57.4 ± 2.6
PBB, 3 mg/kg (n = 8)	432 ± 14	21.4 ± 1.3**	3.5 ± 0.1	57.2 ± 2.8
Control (n = 11)	483 ± 7	16.6 ± 0.3	3.8 ± 0.1	52.7 ± 1.8
PBB, 6 mg/kg (n = 11)	473 ± 8	24.3 ± 0.8*	3.8 ± 0.1	50.7 ± 1.4

[a] Liver wts are repeated from our earlier report (5) for comparative purposes.
[b] Mean ± SE.
* $P < 0.05$.
** $P < 0.01$.

ticular atrophy resulting from high PBB dosages for over 6 weeks in bulls. A physiological alteration in androgen levels in cockerels was indicated by a decrease in comb size and testis weight (10, 11) with PBB administration. In men exposed to PBB there was no effect on spermatogenesis, nor was there any effect on serum gonadotropin or testosterone levels (12). There seem to be no other studies of either plasma testosterone or gonadotropin measurements in PBB-treated subjects. The possibility that longer PBB treatment would interfere with testosterone production or the target organ response to testosterone, as indicated in other studies, cannot be discounted.

PBB administration did not affect pituitary adrenal function as evidenced by the normal diurnal rhythm of plasma corticosterone. Similarly, Garthoff *et al.* (13) reported no change in plasma corticosterone levels after 3 weeks of PBB feeding to rats and PBB ingestion was without effect on adrenal histology (8). In a recent preliminary report, Byrne *et al.* (14) have indicated that both PCB and PBB can decrease plasma corticosterone levels but only after 3–5 months of treatment. The administration of PBB to pregnant rats from Days 7 to 17 resulted in a decrease in adrenal weight (15), although the effect of PBB on pregnancy may have resulted in a secondary adrenal effect.

Following 20 days of PBB administration (3 mg/kg/day), PBB concentration in adrenals was higher than in all other tissues we have examined (e.g., thyroid, liver, and brain) (5, 6), while PBB concentration in the testis was low. After the cessation of PBB administration and a 5-month period of restricted food intake, there was a significant increase in adrenal PBB concentration, in contrast to a decrease in all other

TABLE II. EFFECT OF 20 DAYS OF PBB ADMINISTRATION ON MEAN (± SE) PLASMA LEVELS OF TESTOSTERONE, CORTICOSTERONE, AND PROLACTIN IN MALE RATS

Treatment	Testosterone (ng/dl)	Corticosterone (μg/dl)	Prolactin (ng/ml)
Control	192.1 ± 43.2 (8)[a]	33.9 ± 5.3 (8)	—
PBB (1 mg/kg)	156.8 ± 60.7 (8)	40.1 ± 5.8 (8)	—
Control	203.4 ± 64.8 (8)	37.3 ± 6.5 (8)	60.6 ± 1.7 (8)
PBB (3 mg/kg)	191.6 ± 57.9 (8)	46.3 ± 4.2 (8)	58.6 ± 9.5 (8)
Control	154.6 ± 26.6 (17)	32.6 ± 2.8 (11)	70.1 ± 11.4 (11)
PBB (6 mg/kg)	186.2 ± 21.8 (20)	26.7 ± 3.0 (11)	32.6 ± 6.7 (11)*

[a] Mean ± SE (n).
* $P < 0.005$.

TABLE III. DIURNAL VARIATION IN PLASMA CORTICOSTERONE LEVELS AFTER 20 DAYS OF PBB TREATMENT (3 mg/kg)

	Basal AM corticosterone levels (μg/dl) (0900 hr)	Basal PM corticosterone levels (μg/dl) (1700 hr)
Controls	11.95 ± 2.7 (8)	42.05 ± 6.6 (8)
PBB	17.84 ± 5.5 (8)	30.68 ± 5.4 (8)

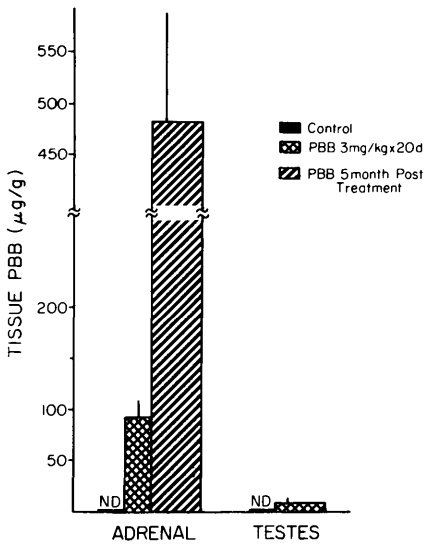

FIG. 1. PBB concentration in adrenal and testis from rats treated with 3 mg/kg PBB for 20 days. PBB were not detectable (ND) in either tissue from control animals. PBB concentration in testis tissue was low (8.7 ± 3.7 μg/g). Concentration in adrenal tissue was high (93.7 ± 15.3 μg/g), and 5 months following treatment with diet restriction was significantly ($P < 0.0025$) increased to 481.1 ± 105.9 μg/g.

tissues examined in this same protocol (5, 6). This increase may represent a mobilization from adipose tissue, which has the highest reported concentration of PBB (16) and a preferential sequestration in the adrenal gland.

At the highest dosage of PBB administered in the present study, a significant reduction of plasma prolactin levels was observed. The depressed prolactin levels may be a result of the hypothyroidism which results from PBB administration (5). A similar depression of prolactin levels has been recorded in surgically hypothyroid rats (17), and represents a decrease in pituitary prolactin content (18).

An interrelationship between thyroid hormone levels and the function of other endocrine organs is well established (19, 20) and it is surprising that the hypothyroidism in PBB-exposed rats did not initiate other endocrine involvement. PBB administration to adult animals in this study had no observed effect on adrenal or testicular functions. PBB have been detected in the milk of lactating mothers (16, 21) and the potential of neonatal exposure to PBB by this route may represent the most critical endocrine involvement (22). It is well known that the developing organism is much more sensitive to a variety of toxic agents than are adults. In addition, maturation of the thyroid axis and its relationship to brain myelination is very important for the final stages of development for neural control of the hypothalamus and pituitary (23). We have demonstrated a significant compromise of thyroid function with PBB administration in the adult and expect that suckling pups may be even more susceptible than adults (22). For the above reasons, the interaction of PBB on thyroid function and endocrine maturation in the neonate represents a most crucial period of PBB action and further studies in this area are required.

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