Effects of Polybrominated Biphenyls on Kidney Function and Activity of Renal Microsomal Enzymes

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Polybrominated biphenyls (PBBs) cause hepatic microsomal enzyme stimulation and histopathological alterations in several organs, including kidney. Concern about effects of PBBs on the health of newborns has increased after the discovery of PBBs in milk of nursing mothers. Therefore, it was of interest to investigate the effects of PBBs on kidney function and the activity of renal microsomal enzymes in adult and immature animals. Seven and eleven day old pups were treated with a single IP injection of either peanut oil or 150 mg/kg PBBs (FireMaster BP-6) in peanut oil. Adult virgin rats were fed diet containing 0 or 100 ppm PBBs for 30 or 90 days. Treatment with PBBs only retarded weight gain after 90 days exposure. Kidney-to-body weight ratio was not altered by PBBs. Arylhydrocarbon hydroxylase activity was increased while epoxide hydratase activity was decreased (adults) or not affected (immature rats) in kidney following treatment with PBBs. Administration of PBBs had no effect on blood urea nitrogen, the clearance of inulin, p-aminohippurate (PAH), or fractional sodium excretion. Similarly, the in vitro accumulation of PAH and N-methyl Nicotinamide (NMN) by thin renal cortical slices and ammoniagenesis and gluconeogenesis in renal cortical slices were not affected by PBBs. In conclusion, treatment with PBBs resulted in modification of the activity of renal microsomal enzyme activities but had no detectable effect on renal function.

Introduction

Polybrominated biphenyls (PBBs) are fire retardants which contaminated livestock and poultry feed in Michigan. The commercial product blended with feed was FireMaster BP-6, which is a mixture of PBB congeners: the major component is 2,2',4,4',5,5'-hexabromobiphenyl (1). Rats maintained on diet containing PBBs have enlarged livers as well as hepatic and renal histopathological alterations (2–4). Renal lesions observed following treatment with 100 ppm octabromobiphenyl include petechial hemorrhage and hyaline degenerative cytoplasmic changes (2, 3). Rat urinary protein is elevated after subchronic administration of PBBs (5). These observations suggest that renal function may be impaired by PBBs.

When administered acutely or subchronically, PBBs increase the activity of hepatic drug metabolizing enzymes in rats (6, 7). Stimulation of hepatic microsomal enzymes following PBBs is similar to that observed after treatment with both phenobarbital (PB) and 3-methylcholanganthrene (3MC) (6), two compounds which represent cytochrome P-450 and P1-450 inducing agents, respectively (8). Currently, there is a scarcity of information regarding PBBs effects on the activity of extrahepatic microsomal enzymes. Increased activity of mixed function oxygenases may alter the metabolism of endogenous compounds and xenobiotics.

Concern about potential effects of PBBs on mothers and offspring has been generated since high concentrations of PBBs have been detected in human milk (9). Young animals may be particularly vulnerable to damage caused by PBBs. The kidney is morphologically and functionally immature at birth and deficient in its capacity to maintain homeostasis when challenged with stressful stimuli. Therefore, it was of interest to investigate the ef-
Effects of PBBs on kidney function and the activity of renal microsomal enzymes in adult and immature animals.

**Methods**

Adult, female virgin and lactating Sprague-Dawley rats and pups (10 animals per litter) (Spartan Farms, Haslett, Mich.) were maintained in clear polypropylene cages at 22°C with a 12-hr light cycle (7 AM-7 PM) and were allowed free access to food (Wayne Lab Blox) and water. Animals were acclimatized for at least 3 days prior to experimental treatment. The 7- and 11-day old pups were treated with a single IP injection of either peanut oil (10 ml/kg) or 150 mg/kg PBBs (FireMaster BP-6, Michigan Chemical Corp., St. Louis, Michigan) in peanut oil, 10 ml/kg. Following acclimation, adult virgin rats were treated by substituting ground diet containing 0 or 100 ppm PBBs (FireMaster BP-6) for the Lab Blox. Rats were exposed to the diet for 30 or 90 days.

Enzyme assays were performed 28 days following injection of seven day old pups and after 90 days exposure of adult rats to the experimental diet. Immediately following sacrifice, kidneys were excised, weighed and then chopped into ice-cold 66mM Tris buffered to pH 7.4 with HCl. Renal postmitochondrial supernatants were prepared by homogenization (Potter-Elvehjem homogenizer with a Teflon pestle) in 3 volumes of 66mM Tris pH 7.4 followed by centrifugation at 10,000g for 30 min. All assays were performed on the day of supernatant preparation. Protein was measured (10) by use of bovine serum albumin as a standard. Enzyme activities measured were epoxide hydratase (EH) (11) and arylhydrocarbon hydroxylase (AHH) (12, 13).

Animals used for histologic examination had been exposed to the experimental diet for 90 days. Pieces of kidney were cut and fixed in 10% buffered formalin. After fixation, these were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin.

Organic ion transport capacity was estimated in vitro 3 days following injection of 11-day-old pups and after 30 and 90 days exposure of adult rats to the experimental diet. Following sacrifice, kidneys were quickly removed, weighed, and placed in ice-cold normal saline. Transport capacity was quantified as the ability of renal cortical slices to accumulate a representative anion, p-aminohippuric acid (PAH), and cation, N-methyl nicotinamide (NMN) (14, 15). Results were expressed as slice-to-medium (S/M) concentration ratio, where S is in units of mg/g wet tissue weight and M is expressed in mg/ml medium. The ability of renal cortical slices from rats treated with PBBs for 90 days to form ammonia and glucose was also determined (16, 17). Net production of ammonia and glucose was expressed as µmole/mg wet tissue weight/hr.

To determine renal function in vivo, animals exposed to PBBs for 30 or 90 days were anesthetized with 50 mg/kg sodium pentobarbital, IP, and body temperature was maintained at 36–38°C by use of heat lamps. A PE50 cannula was inserted into the bladder, and urine was collected under mineral oil in preweighed vials. The left femoral vein was cannulated for infusion. Both femoral arteries were cannulated to monitor blood pressure and to obtain blood samples. The infusion solution contained 1% inulin and 0.6% PAH in normal saline. (3H)-Inulin (0.5 µCi/ml) and (14C)-PAH (0.5 µCi/ml) were added to the solution, which was infused at 0.019 ml/min using a Harvard infusion pump. A minimum of 1.5 hr elapsed from the beginning of the infusion to initiation of urine collection. The 30-min urine collections were taken with blood (400 µl) sampled at the middle of each period. (14C)-PAH, (3H)-inulin, and sodium concentrations were determined as previously described (18). Blood urea nitrogen (BUN) was quantified (17) and expressed as weight (mg) urea nitrogen per 100 ml of whole blood.

Data were analyzed statistically by randomized complete block analysis of variance. Treatment differences were detected by the least significant difference test (19). The 0.05 level of probability was used as the criterion of significance.

**Results**

Growth rate was not affected by a single IP injection of 150 ppm (150 mg/kg) PBBs or by 30 days treatment with 100 ppm PBBs; however, 90 days dietary exposure to 100 ppm PBBs retarded weight gain (Table 1). The kidney to body weight ratio was not altered by treatment with PBBs (Table 1).

Table 1. Effect of PBBs on body weight and kidney to body weight ratio.

| Treatment time, days | PBB Treatment, ppm | Body weight, g<sup>a</sup> | Kidney weight/ body weight<sup>a</sup> |
|----------------------|-------------------|---------------------------|----------------------------------|
| 30                   | Control 100       | 234.2 ± 6.9              | 0.78 ± 0.07                      |
|                      | 100               | 221.5 ± 8.3              | 0.75 ± 0.04                      |
| 90                   | Control 100       | 276.7 ± 4.8              | 0.71 ± 0.02                      |
|                      | 150               | 250.2 ± 5.4<sup>b</sup>  | 0.76 ± 0.04                      |
| Acute<sup>c</sup>    | Control 100       | 121.4 ± 1.3              | 1.05 ± 0.04                      |
|                      | 150               | 117.2 ± 0.7              | 1.03 ± 0.08                      |

<sup>a</sup> Values are means ± S.E.M. for four animals.

<sup>b</sup> Statistically significant difference from the respective control, p < 0.05.

<sup>c</sup> Seven-day-old rats were given a single treatment with PBBs and sacrificed 28 days later.
Administration of 150 ppm PBBs to 7-day-old rats increased the activity of renal arylhydrocarbon hydroxylase (AHH) above controls 28 days later (54% of control). Epoxide hydratase (EH) activity was not modified in kidneys of young rats by PBBs. The activity of renal AHH in rats exposed to 100 ppm PBBs for 90 days was increased above control (1116% of control), however, renal EH activity was significantly decreased (14% of control) (Table 2).

Table 2. Effect of PBBs on renal AHH and EH activities.

| Treatment | PBB treatment, ppm | AHH, fluorescence | EH, n mole styreneglycol formed/ | S/M ratio<sup>a</sup> |
|-----------|--------------------|-------------------|---------------------------------|---------------------|
| time, days|                    | units/mg microsomal protein-min<sup>a</sup> | mg microsomal protein-min<sup>a</sup> | PAH                 | NMN                 |
| 90        | Control 100        | 2.94 ± 1.35       | 0.14 ± 0.06                      |                    |
| 90        | Acute<sup>c</sup>  | 100               | 32.82 ± 1.73<sup>a</sup>         | 0.02 ± 0.01<sup>a</sup> |
|           |                    | 150               | 4.39 ± 2.36                      | 0.29 ± 0.02        |
|           |                    | 150               | 24.08 ± 8.22<sup>a</sup>         | 0.33 ± 0.03        |

<sup>a</sup> Values are means ± S.E.M. for three animals.
<sup>b</sup> Statistically significant difference from the respective control, p < 0.05.
<sup>c</sup> Seven-day-old rats were given a single treatment with PBBs and sacrificed 28 days later.

Treatment with PBBs had no effect on the in vitro accumulation of PAH and NMN by thin renal cortical slices (Table 3) or ammoniagenesis and gluconeogenesis by renal slices (Table 4).

The clearance of inulin (glomerular filtration rate, GFR), the clearance of PAH (effective renal plasma flow, ERPF) and fractional sodium excretion (%) were unaffected by 30 or 90 days exposure to diet containing 100 ppm PBBs (Table 5). Similarly, administration of PBBs for 30 or 90 days had no effect on blood urea nitrogen (BUN) (Table 6).

Table 3. Effect of PBBs on the accumulation of PAH and NMN.

| PBB treatment, ppm | S/M ratio<sup>a</sup> |
|--------------------|-----------------------|
| 100                | PAH: 10.17 ± 1.46     | NMN: 5.71 ± 0.40               |
| 150                | PAH: 9.84 ± 0.87      | NMN: 5.59 ± 0.22               |
| 90 Days            |                        |                                   |
| Control 100        | PAH: 10.69 ± 0.67     | NMN: 5.79 ± 0.10                |
| 90 Days            |                        |                                   |
| 150                | PAH: 10.16 ± 0.76     | NMN: 5.20 ± 0.17                |
| Acute<sup>b</sup>  |                        |                                   |
| Control 100        | PAH: 8.29 ± 0.81      | NMN: 5.10 ± 0.31                |
| 150                | PAH: 8.56 ± 0.33      | NMN: 5.63 ± 0.17                |

<sup>a</sup> Values are means ± S.E.M. for at least three animals.
<sup>b</sup> Two-week-old rats were given single treatment with PBBs 72 hr prior to sacrifice.

Table 4. Effect of feeding PBPB for 90 days on ammoniagenesis and gluconeogenesis.

| PBB treatment, ppm | Net production, μmole/mg wet tissue weight/hr<sup>a</sup> | S/M ratio<sup>a</sup> |
|--------------------|----------------------------------------------------------|-----------------------|
|                    | Ammonia | Glucose | PAH | NMN |
| Control 100        | 3.39 ± 0.09 | 0.03 ± 0.01 | 5.71 ± 0.40 | 5.59 ± 0.22 |
| 100                | 3.45 ± 0.09 | 0.03 ± 0.01 | 5.10 ± 0.31 | 5.63 ± 0.17 |

<sup>a</sup> Values are means ± S.E.M. for four animals.

**Discussion**

Polybrominated biphenyls stimulate hepatic microsomal mixed function oxygenases in adult rats and mice (6, 7, 20). The results of this investigation indicate that PBBs also alter the activity of renal microsomal enzymes in immature and adult rats. Activity of AHH is increased in kidney as in liver after PBBs; however, in contrast to hepatic EH stimulation, renal EH activity is decreased (adults) or not affected (immature rats) by treatment with PBBs. Stimulation of AHH with concomitant inhibition of EH in kidney may be of toxicological concern. Microsomal enzymes such as AHH can catalyze the formation of very reactive arene oxides from inert aromatic compounds (21, 22). Arene oxides may be further metabolized to less toxic dihydrodiols by enzymes such as EH (22). Thus, since AHH activity is increased while EH activity is...
Table 5. Effect of feeding PBBs on glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and fractional sodium excretion.

| Treatment time, days | GFR, ml/min<sup>a</sup> | ERPF, ml/min<sup>a</sup> | Fractional sodium excretion, % |
|----------------------|--------------------------|--------------------------|-------------------------------|
|                      | Control 100 ppm PBBs     | Control 100 ppm PBBs     | Control 100 ppm PBBs          |
| 30                   | 2.78 ± 0.19 2.42 ± 0.57  | 9.47 ± 0.54 9.01 ± 0.32  | 0.14 ± 0.06 0.29 ± 0.11       |
| 90                   | 2.46 ± 0.16 2.34 ± 0.21  | 10.87 ± 0.63 8.77 ± 0.97  | 0.17 ± 0.04 0.12 ± 0.03       |

<sup>a</sup> Values are means ± S.E.M. for four animals.

Table 6. Effect of feeding PBBs on blood urea nitrogen.

| PBB treatment, ppm | Duration, days | BUN, mg urea nitrogen/100 ml/blood<sup>a</sup> |
|--------------------|----------------|-----------------------------------------------|
| Control            | 30             | 17.28 ± 2.75                                  |
| 100                | 30             | 22.19 ± 1.92                                  |
| Control            | 90             | 19.06 ± 1.86                                  |
| 100                | 90             | 18.59 ± 1.52                                  |

<sup>a</sup> Values are means ± S.E.M. for four animals.

Acute and subchronic administration of PBBs to immature and adult rats, respectively, modified the activity of renal microsomal enzymes. Alterations in enzyme activities were not correlated with changes in renal function. This indicates that PBBs, at least FireMaster BP-6, may not be a potent nephrotoxic agent. However, the characteristics of mixed function oxygenase alteration in kidney following treatment with PBBs suggest that this compound may predispose the kidney to toxicity produced by chemicals administered subsequent to PBBs.

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