INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY POLYCHLORINATED BIPHENYLS IN THE FOETO-PLACENTAL UNIT AND NEONATAL LIVERS DURING LACTATION

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1. Introduction

Polychlorinated biphenyls (PCBs) have been widely used as industrial chemicals in a variety of commercial and domestic products. They also constitute a major component of microscope immersion oils. Residues of PCBs have been widely found in tissues of fish and wild life and contamination of human adipose tissue [1,2] and of human milk [3] has been previously reported. Recent studies from this laboratory [4] have shown that PCBs are potent inducers of the hepatic mixed function oxidase system, enhancing the metabolism of a variety of substrates and causing marked increases in the concentration of cytochrome *P*-450, effects similar to the barbiturate class of inducing chemicals. PCBs also cause several fold increases in hepatic aryl hydrocarbon hydroxylase [4], the enzyme involved in the metabolism of chemical carcinogens, such as benzo(a)pyrene. In this respect, PCBs share a number of properties of the carcinogen type of inducers of microsomal enzymes, including that of inducing the synthesis of cytochrome *P*-448 [4].

Since PCBs have been found in human adipose tissue and in human milk, it was of interest to determine the effects of these chemicals on the fetal environment as well as their possible effects on neonatal liver enzymes as mediated by the transmission of these chemicals through maternal milk. In this regard, the effects of the PCBs were compared to those of phenobarbital and the polycyclic hydrocarbon carcinogen, 3-methylcholanthrene (3-MC).

2. Experimental

Sprague-Dawley pregnant rats were injected intraperitoneally with either 3-MC, 25 mg/kg/day for 4 days, or phenobarbital, 37.5 mg/kg twice daily for 4 days or PCBs, 25 mg/kg/day for 6 days. The PCB mixture used was Aroclor 1254 (Monsanto Chemical Co.). All rats were sacrificed 24 hr after the last injection. The day of sacrific was the 20th day of pregnancy. In the lactation experiments, similar treatments were instituted post-partum to previously untreated mothers starting on day 2 after birth for the PCBs and day 4 after birth for phenobarbital and 3-MC. Neonates of these mothers were sacrificed on day 8.

Placentas and fetal livers from each pregnant rat were pooled. Similarly, livers from the neonates of each mother were pooled for the enzyme assays. Benzo(a)pyrene hydroxylase activities were determined on whole homogenates as described previously [5] using incubation times and protein concentrations such that reaction rates were linear with the different tissue preparations. Ethylmorphine *N*-demethylase [6] and cytochrome *P*-450 contents [7] were determined on the microsomal fractions as described previously. Protein was determined by the Lowry method [8].

3. Results

The effects of administration of PCBs, 3-MC or phenobarbital to pregnant rats on benzo(a)pyrene

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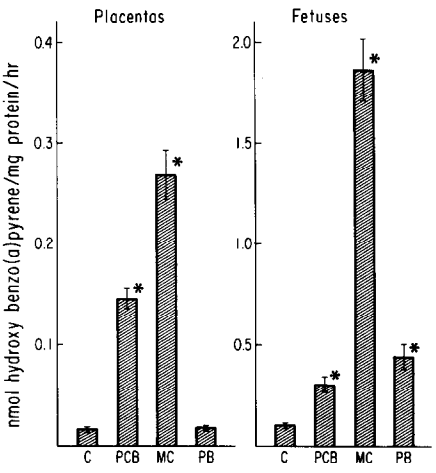

Fig.1. Induction of benzo(a)pyrene hydroxylase in 20-day old placentas and fetal livers by polychlorinated biphenyls PCB, 3-methylcholanthrene MC and phenobarbital PB. Enzyme activity was determined in pooled placentas and in pooled livers of fetuses from each rat. Each value represents the mean ± S.E. obtained from 5 pregnant rats. Asterisks represent values significantly different from the control (C) group ($P<0.05$).

hydroxylase activities in placentas and fetal livers are shown in fig.1. PCBs caused a 10-fold induction of the hydroxylase activity in the placenta but only a 3-fold induction in the fetal livers. 3-MC, on the other hand caused an 18- to 20-fold induction in both the placental and the fetal hydroxylase activities. Phenobarbital pretreatment resulted in no significant increases in placental hydroxylase activity but caused a 4-fold increase in fetal hydroxylase activity similar in magnitude to that observed with the PCBs. It should be noted that control values of the enzyme in placentas of untreated rats were markedly lower than those observed in the fetal livers of the same rats.

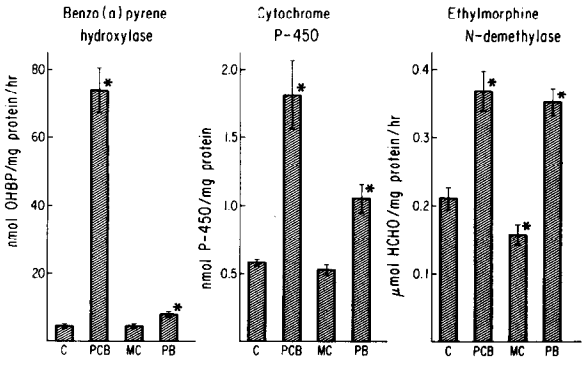

Fig.2. Induction of neonatal liver microsomal enzymes by polychlorinated biphenyls PCB, 3-methylcholanthrene MC and phenobarbital PB transmitted through maternal milk. Livers from the neonates of each mother were pooled for the enzyme assays. Each value represents the mean ± S.E. obtained from neonates of 5 mothers. Asterisks represent values significantly different from the control (C) group ($P<0.05$).

The effects of treating mother rats with PCBs, 3-MC or phenobarbital on microsomal enzymes in the neonatal liver are shown in Fig.2. The experimental neonates were thus exposed to these chemicals from the mother by suckling. The control values observed with the neonatal livers are lower than those obtained with adult livers. PCBs administered to the mother had the greatest effect: an 18-fold increase in benzo(a)pyrene hydroxylase activity, a 3-fold increase in cytochrome *P*-450 content and a 2-fold increase in *N*-demethylase activity. The CO-difference spectrum of cytochrome *P*-450 showed a shift in the absorption maximum from 450 to 499 nm in the PCB exposed neonates, indicating that the hemoprotein induced may be a mixture of cytochromes *P*-450 and *P*-448. 3-MC treatment of the mothers resulted in no increases in any of the enzymes studied. In contrast, 3-MC caused a small but significant decrease in *N*-demethylase activity. Treatment of the mothers with phenobarbital resulted in less than 2-fold increases in cytochrome *P*-450 and in *N*-demethylase and hydroxylase activities in the livers of the neonates.

4. Discussion

The present study demonstrates that the administration of PCBs and 3-MC to pregnant rats induces the aryl hydrocarbon hydroxylase activity in the placenta as well as in the fetus. The degree of induction of the placental and the fetal liver enzymes by 3-MC appeared to be similar, as has been previously reported [9]. However the response to PCB induction was greater in the placenta than in the fetal liver. Phenobarbital caused a small but significant increase in benzo(a)pyrene hydroxylase activity in fetal liver, but not in placenta.

When these chemicals were administered to neonates via the maternal milk, PCBs were the most potent of the three, causing an 18-fold increase in benzo(a)py-

rene hydroxylase activity, a 3-fold increase in cytochrome *P*-450 and a 2-fold increase in *N*-demethylase activity in the neonatal liver. 3-MC administration resulted in no stimulation of these enzymic activities. Phenobarbital treatment resulted in less than 2-fold increases in the microsomal enzymes assayed.

These data show that although PCBs and 3-MC are both highly lipid soluble chemicals, 3-MC had a greater effect on the foeto-placental unit than PCBs; whereas the PCBs were more potent inducers in neonatal liver, when the neonate received the chemicals through mother's milk. These data may indicate differences in membrane permeability, protein binding capacities or the interaction of these chemicals with humoral factors in the placenta or the residual effects of these factors in the neonate. Whatever the mechanistic differences may be, it is evident that PCBs can be transmitted through maternal milk and since they have already been found in human milk [3] these findings suggest the possibility that the levels of these enzymes may be altered in neonates so exposed. The induction of the aryl hydrocarbon hydroxylase system by the PCBs may be of particular importance in chemical carcinogenesis since recent studies [10] have shown that inducibility of lymphocyte aryl hydrocarbon hydroxylase was significantly greater among cigarette smokers with bronchogenic carcinoma than among healthy non-smoking control subjects.

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