Pergamon

0960-0760(95)00060-7

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 6, pp. 595–597, 1995

0960-0760/95 $9.50 + 0.00

Effect of PCBs on Androgen Production by Suspension of Adult Rat Leydig Cells *in vitro*

R. Kovačević,[1]* M. Vojinović-Miloradov,[2] I. Teodorović[1] and S. Andrić[1]

[1]Institute of Biology and [2]Institute of Chemistry, Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, Serbia

The effects of PCBs (mixture of 2, 3, 4, 5-tetra; 2, 2′, 4, 5, 5′-penta; 2, 2′, 3, 3′, 6, 6′-hexa and 2, 2′, 3, 3′, 4, 4′, 5, 5′-octa congeners) on androgen production were investigated by suspension of Leydig cells from adult rat testis. hCG-stimulated androgen production was significantly inhibited by PCBs while progesterone level was not affected. Progesterone supported testosterone production was also decreased by PCBs, while conversion of androstenedione to testosterone was unchanged. These results suggest that the activity of microsomal enzyme C21 side-chain cleveage *P*450 was decreased by PCB treatment of Leydig cells *in vitro*.

J. Steroid Biochem. Molec. Biol., Vol. 52, No. 6, pp. 595–597, 1995

INTRODUCTION

The polychlorinated biphenyls (PCBs) have been widely identified as environmental pollutants. Their industrial use lasted from the early '30s until the 1980s when they were banned. However, they are still entering the environment in several ways [1, 2]. The results concerning toxic effects of PCBs on mammalian reproduction are enlarged [3–9]. Studies in rats have shown that a single *in vivo* dose of the coplanar PCB isomer, 3, 4, 5, 3′, 4′, 5′-hexachlorobiphenyl produced a severe reduction in the plasma testosterone concentration [3]. Also, the postnatal exposure of male rats to PCBs caused impaired reproductive function in adults [4]. However, lack of the effect of PCBs on plasma testosterone levels, as well as on *in vitro* androgen production was demonstrated in the mouse [5]. There is ample evidence that PCBs could influence reproductive function in females [6–9].

Since the effects on reproduction may be attributed to alterations of steroid-metabolizing enzymes, the aim of the present study was to investigate the effects of PCBs on hCG-stimulated androgen production by suspension of Leydig cells from adult rat testis. In order to determine the possible site of PCBs action on the steroidogenic enzymes, the effect on testosterone production supported by progesterone or androstenedine as a substrate was also investigated.

*Correspondence to R. Kovačević.
Received 19 Sep. 1994; accepted 18 Jan. 1995.

EXPERIMENTAL

Chemicals

PCB mixture (stock solution; PCBs dissolved in toluol) contained tetra (2, 3, 4, 5; 0.01 mg/ml), penta (2, 2′, 4, 5, 5′; 0.01 mg/ml), hexa (2, 2′, 3, 3′, 6, 6′; 0.007 mg/ml) and octa (2, 2′, 3, 3′, 4, 4′, 5, 5′; 0.022 mg/ml) congeners and was kindly donated by Dr B. Webster (University of Manitoba, Canada) and antitestosterone serum No. 250 by Dr G. D. Niswender (Colorado State University, U.S.A.). Medium 199 (M199), bovine serum albumine (BSA, Fraction V), collagenase (Type I), testosterone, progesterone, androstenedione and antiprogesterone serum (raised against progesterone-11α-BSA) were purchased from Sigma; hCG (Pregnyl, 3000 IU/mg) was obtained from Organon Inc.; and [1, 2, 6, 7-^{3}H(N)]progesterone and [1, 2, 6, 7-^{3}H(N)]testosterone were obtained from New England Nuclear. All other reagents were of analytical grade.

Animals and cell dispersion

Male Wistar rats, random bred in our laboratory, were raised under controlled environmental conditions, with food and water *ad libitum*. Adult rats (around 3 months old) were killed by decapitation, testes quickly removed, decapsulated and enzymatically dispersed with collagenase as previously described [10]. A trypan blue exclusion test was used to determine total cell counts and viable cell number.

Testosterone production in vitro

Aliquots (10^6 cells/0.2 ml) of crude cell suspension of Leydig cells were added to 12 × 75 ml plastic tubes containing 0.1 ml of hCG (10 ng) or steroid substrates (2 μM) with or without 0.2 ml of PCB mixture so that final volume was 0.5 ml. The desired concentration of PCBs was prepared by evaporating the necessary amount of stock solution and dissolving it in the medium 199 enriched by BSA (0.1%), (M199-BSA). All tubes were incubated for 2 h in a shaking water bath oscillating at 100 cycles/min under an atmosphere of 95% O_2–5% CO_2. The tubes were centrifuged for 5 min at 400 *g* at 4°C and the supernatant was stored at −20°C prior to measurement of testosterone and progesterone by RIA. Since the anti-testosterone-11-BSA serum No. 250 (from G. D. Niswender) shows high cross-reactivity with dihydrotestosterone (and not with other steroids), assay values for androgen production are referred to as "T + DHT" concentrations. The pelleted cells in the tube were resuspended in M199-BSA (0.4 ml/tube), and the cell viability was assessed by trypan blue exclusion test.

RESULTS

The results shown in Table 1, indicate that PCB mixture induced a dose-dependent decrease of Leydig cell viability after 2 h incubation. Since in the presence of the lowest dose of PCBs (5×10^{-6} M), the number of viable cells after 2 h incubation was not altered that dose of PCBs was used in the later experiments. PCBs inhibited androgen production in the presence of saturated dose of hCG, while progesterone levels were unaffected (Table 2). Conversion of the individual metabolites of the microsomal part of the steroidogenic pathways (progesterone, androstenedione) to testosterone was tested in the presence of 5×10^{-6} M of PCBs (Table 3). An inhibitory effect on testosterone production was observed if progesterone was used as a substrate. However, conversion of androstenedione was not affected.

Table 1. Effect of different doses of PCBs on viability of adult rat Leydig cells after 2 h incubation

Group	PCBs (M)	Percent of viable cells after 2 h incubation
Controls	—	100
PCBs	5.20×10^{-6}	100
	1.04×10^{-5}	71.03
	1.56×10^{-5}	42.48
	2.08×10^{-5}	2.20

Leydig cells (1×10^6) were incubated for 2 h in the presence of different concentrations of PCBs (5 replicates for each group). At the end of incubation the tubes were centrifuged at 400 *g*, pelleted cells were resuspended in 0.4 ml of M199-BSA (combined together for each group) and the cell viability was assessed by Trypan blue exclusion test.

Table 2. Effect of PCBs on hCG-stimulated production of progesterone and androgen (T + DHT) by suspension of adult rat Leydig cells

Group	Production of progesterone (pg/10^6 L.c.)	Production of T + DHT (ng/10^6 L.c.)
Controls	131.75 ± 6.11 (5)	16.39 ± 1.03 (5)
PCBs	137.97 ± 20.20 (4)	11.94 ± 1.25 (4)*

Leydig cells (1×10^6) were incubated for 2 h in the presence of a saturated dose of hCG (10 ng/10^6 L.c.) with or without PCBs (5.2×10^{-6} M). Numbers represent mean ± SEM. Numbers in brackets represent number of replicates. Significance: *$P < 0.05$ vs control. Results are representative of 3 experiments. L.c., Leydig cells.

DISCUSSION

In the present study, the inhibitory effect of PCBs on hCG-stimulated androgen production by suspension of rat Leydig cells has been recorded. Since the progesterone level in these incubations was unchanged, the inhibitory effect at the level of microsomal enzymes would be expected. Such an effect was confirmed by decrease of progesterone supported testosterone production when Leydig cells were exposed to PCBs. On the other hand, the conversion of androstenedione to testosterone was not changed by PCBs treatment. According to our results, it could be supposed that PCBs decrease the activity of C21 side-chain cleavage *P*450, which is known to catalyze 17α-hydroxylation and C17, 20 lyase activity [11]. However, the results of Goldman and Yawetz [12] have shown that Aroclor 1254 induced no changes in the activity of 17α-hydroxylase/C17 lyase in guinea pig testis microsomes, either of pretreated animals or when Aroclor 1254 (13.3 μM) was present in the reaction preparations.

At the present moment it is not possible to give a clear explanation for such a discrepancy. However, the differences in PCB congeners contained in Aroclor

Table 3. Effect of PCBs (5.2×10^{-6} M) on androgen (T + DHT) production by suspension of adult rat Leydig cells in the presence of progesterone (2 μM) and androstenedione (2 μM) as substrate

Substrate	Levels of T + DHT (ng/10^6 L.c.)	
	Controls	PCBs
Progesterone	32.59 ± 1.72 (5)	25.85 ± 1.91 (5)*
Androstenedione	80.70 ± 10.25 (4)	68.83 ± 4.83 (5)

Leydig cells (1×10^6) were incubated for 2 h in the presence of progesterone or androstenedione, with or without PCBs (5.2×10^{-6} M). Numbers represent mean ± SEM. Numbers in brackets represent number of replicates. Significance: *$P < 0.001$ vs. control. Results are representative of 2 experiments. L.c., Leydig cells.

1254 (polychlorobiphenyl containing 54% chlorine; tetra, 11%; penta, 49%; hexa, 34%; and hepta-congeners, 6%) and in our mixture (high amount of octa-congeners, 49%) could account for such results. Also, the differences in the test model used (microsomal preparations or whole cell system) would not be neglected.

According to the presented data the mechanism by which PCBs decreased the activity of C21 side chain cleveage *P*450 could not be precisely defined. Further experiments are necessary to clarify if the mixture of PCBs act directly on the enzyme and/or there is an indirect effect through a change in the hydrophobic environment in which the enzyme is embedded. Several reports have shown that the changes in membrane phospholipid composition could alter the activity of some rat testicular enzymes [13–15].

Our data also showed that higher doses of PCBs ($\geqslant 10^{-5}$ M) decrease cell viability after 2 h incubation, which indicated the toxic effect of PCBs on plasma membrane integrity. However, the dose of 5×10^{-6} M which altered androgen production of Leydig cells, had no effect on cell viability. That dose corresponds to the range of 1–2 ppm.

Considering the results from our study, together with the data from the literature [3–9, 14, 16] it could be supposed that PCBs are environmental pollutants with certain negative influence on reproductive function.

Acknowledgements—We are grateful to Dr B. Webster for the gift of PCBs and to Dr G. D. Niswender for the supply of antiserum. This research was supported by a joint grant from the Serbia Research Association.

REFERENCES

1. Ahlborg U. G., Brouwer A., Fingerhut M. A., Jacobson J. L., Jacobson S. W., Kennedy S. W., Kettrup A. A. F., Koeman J. H., Poiger H., Rappe C., Safe S. H., Seegal R. F., Tuomisto J. and van den Berg M.: Impact of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. *Eur. J. Pharmac. Eur. Toxic. Pharm. Sec.* **228** (1992) 179–199.
2. Vojinović-Miloradov M., Marjanović P., Buzarov D., Pavkov S., Dimitrijević Lj. and Miloradov M.: Bioaccumulation of polychlorinated biphenyls and organochlorine pesticides in selected fish species as an indicator of the pollution of aquatic resources in Voivodina, Yugoslavia. *W. Sci. Tech.* **26** (1992) 2361–2364.
3. Yeowell H. N., Waxman D. J., Wadhera A. and Goldstein J. A.: Suppression of the constutive, male specific rat hepatic cytochrome *P*-450 2c and its mRNA by 3, 4, 5, 3′, 4′, 5′-hexachlorobiphenyl and 3-methylcholanthrene. *Molec. Pharmac.* **32** (1987) 340–347.
4. Sager D. B.: Effect of postnatal exposure to polychlorinated biphenyls on adult male reproductive function. *Environ. Res.* **31** (1983) 76–94.
5. Johansson B.: Lack of effects of polychlorinated biphenyls on testosterone synthesis in mice. *Pharmac. Toxic.* **61** (1987) 220–223.
6. Brezner E., Terkel J. and Perry A. S.: The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat. *Comp. Biochem. Physiol.* **77C** (1984) 65–70.
7. Smitsvabprooije A. E., Lammers J. H. C. M., Waalkemsberendsen D. H., Kulig B. M. and Snoij N. J.: Effects of the PCB 3, 4, 5, 3′, 4′, 5′-hexachlorobiphenyl on the reproduction capacity of Wistar rats. *Chemosphere* **27** (1993) 395–400.
8. Jonsson H. T., Keil J. E., Gaddy R. G., Loadholt C. B., Henniger G. R. and Walker E. M.: Prolonged ingestion of commercial DDT and PCB: effects on progesterone levels and reproduction in the mature female rat. *Arch. Environ. Contam. Toxic.* **3** (1976) 279–290.
9. Backlin B. M. and Bergman A.: Morphological aspects on the reproductive organs in female mink (Mustela Vison) exposed to polychlorinated biphenyls and fractions thereof. *Ambio* **21** (1992) 596–601.
10. Kovačević R. and Sarač M.: Bromocriptine-induced inhibition of hydroxylase/lyase activity of adult rat Leydig cells. *J. Steroid Biochem. Molec. Biol.* **46** (1993) 841–845.
11. Nakajin S., Shikita M. and Hall P. F.: Microsomal cytochrome *P*450 from neonatal pig testis: purification and properties of a C21 side-chain cleavage system (17-hydroxylase-C17-20 lyase). *J. Biol. Chem.* **256** (1981) 3871–3876.
12. Goldman D. and Yawetz A.: The interference of Aroclor 1254 with progesterone metabolism in guinea pig adrenal and testes microsomes. *J. Biochem. Toxic.* **5** (1990) 99–107.
13. Bogovich K. and Payne A. H.: Purification of rat testicular microsomal 17-ketosteroid reductase. Evidence that 17-ketosteroid reductase and 17β-hydroxysteroid dehydrogenase are distinct enzymes. *J. Biol. Chem.* **260** (1980) 5552–5559.
14. Cooke G. M. and Robaire B.: Modulation of epididymal Δ^4-steroid 5α-reductase activity *in vitro* by the phospholipid environment. *J. Biol. Chem.* **260** (1985) 7489–7495.
15. Cooke G. M. and Robaire B.: Phospholipases modulate the rat testicular androgen biosynthetic pathway *in vitro*. *Biol. Reprod.* **39** (1988) 329–339.
16. Miranda C. L., Henderson M. C., Wang J. L., Chang H. S., Hendricks J. D. and Buhler D. R.: Differential effects of 3, 4, 5, 3′, 4′, 5′-hexachlorobiphenyl (HCB) on interrenal steroidogenesis in male and female rainbow trout *Oncorhynchus Mykiss*. *Comp. Biochem. Physiol.* **103C** (1992) 153–157.