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In vivo and *in vitro* effects of PCB126 and PCB153 on rat testicular androgenesis

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Abstract

In this study we compared the effects of PCB126 and PCB153 on adult rat testicular androgenesis and the status of antioxidant enzymes in the interstitial cell compartment 96 h after local intratesticular application. Obtained results indicated PCB126-induced inhibition of conversion of progesterone (P) and Δ^4-androstenedione (A^4) to testosterone (T), and stimulation of conversion of P to T induced by PCB153, while combined application had no effect. Activities of antioxidant enzymes were unchanged, except of decreased activity of SOD in PCB126-treated group. In parallel experiments, adult purified Leydig cells challenged with PCB congeners were incubated for 2 h in the presence of corresponding steroid substrates. Results demonstrated that in the presence of subsaturating substrate concentrations PCB126 induced inhibition of conversion of P and A^4 to T at nM to μM doses, while PCB153 caused stimulation at nM concentrations. Further studies should indicate possible mechanism(s) of modulation of androgenesis by tested PCB congeners.

Keywords: PCB126; PCB153; Rat Leydig cells; Androgenesis

1. Introduction

Polychlorinated biphenyls (PCB) are the man-made chemicals that may disrupt gonadal functions by altering steroid hormone production and metabolism, acting as agonists for steroid hormone receptors and/or changing the level of receptor expression (Cooke et al., 2001). Our previous research has been focused on the effects of commercial PCB mixture and/or PCB-based transformer fluid on testicular androgenesis and activity of certain steroidogenic enzymes in adult rat testis. Obtained results indicate strong inhibition of testicular androgenesis 24 h after *in vivo* and 10–15 min after *in vitro* exposure to Aroclor 1248 and PCB-based transformer fluid (Andric et al., 2000a,b, 2001). However, there is limited information about the effects of individual PCB congeners on gonadal steroidogenesis (Wojtowicz et al., 2000, 2005; Fukazawa et al., 2003). It is known that PCBs also affect two enzyme systems responsible for production and elimination of reactive intermediates: the xenobiotic metabolizing enzymes (XMEs) and antioxidant enzymes (AOEs) (Fukazawa et al., 2003; Twarowski et al., 2001, Murugesan et al., 2005, 2007). When induced, CYP enzymes may serve as a source of reactive oxygen species (ROS). In line with this, during altered steroidogenesis ROS are also produced through CYP enzymes controlling steroidogenesis, and therefore testes possess strong antioxidant defence (Peltola et al., 1994). Our previous study (Andric et al., 2003) showed that changes in oxidative status in testicular milieu do not necessarily correlate with disruption of steroidogenesis in PCB-treated rats. However, changes in the activities of any of the enzyme system mentioned above could influence testosterone output from Leydig cells.

In this study we compared the effects of individual congeners, PCB126 (3,3′,4,4′,5-PCB) and PCB153 (2,2′,4,4′,5,5′-PCB) on rat testicular steroidogenesis, activity of AOEs in the interstitial testicular cells, and XMEs in testis and liver 96 h after bilateral intratesticular injection of PCBs. Direct *in vitro* effect of these two congeners on purified Leydig cell androgenesis was also investigated. Obtained results demonstrated similar pattern of *ex vivo* and *in vitro* differences in PCB126- and PCB153-induced modulation of rat testicular androgenesis.

2. Material and methods

2.1. Chemicals

PCB126 (3,3′,4,4′,5-PCB), and PCB153 (2,2′,4,4′,5,5′-PCB) were purchased from AccuStandard Inc. (New Haven, CT, USA). The

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antitestosterone-11-bovine-serum albumin (BSA) serum #250 was supplied by Dr. Niswender (Colorado State University, Fort Collins, CO, USA). [1,2,6,7,3H(N)]-Testosterone was obtained from New England Nuclear (Brussels, Belgium), whereas NADPH, BSA (fraction V), collagenase (type IA), collagenase (type IV), ethoxyresorufin, pentoxyresorufin, resorufin, glucose-6-phosphate dehydrogenase, glucose-6-phosphate, testosterone, Medium 199 (M199), DMEM/F12, Hepes and Percoll were obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Animals and in vivo treatments

All experiments were performed in adult male Wistar rats raised in our vivarium under controlled environmental conditions (temperature 22 ± 2 °C and 14 h light/10 h dark) with food and water *ad libitum*. Rats were handled daily during one-week acclimation period prior to experiments. Groups of rats were treated with single bilateral intratesticular injection of two doses of PCB126 (0.1 nmol/testis and 1 nmol/testis) and PCB153 (10 nmol/testis and 100 nmol/testis). Applied PCB congener doses were in the range of their presence in the PCB-based transformer oil (PCB at 2.3%, and PCB153 at 23%; as described in Andric et al., 2006) used in our previous studies (Andric et al., 2000a, 2003). The substances were dissolved in saline to desired concentration after evaporating the required amount of stock solution. Animals were sacrificed 96 h after injection. All experiments were approved by Local Animal Ethical Committee of the University of Novi Sad and were conducted in accordance with the principles and procedures of the NIH Guide for Care and Use of Laboratory Animals.

2.3. Ex vivo androgenesis

The 17β-hydroxysteroid dehydrogenase (17βHSD) and CYP 17 activities were measured as previously described (Kostic et al., 2000), and were estimated by conversion of Δ^4-androstenedione (A^4) to testosterone (T) and progesterone (P) to T, respectively, except that microsomal fractions, rather than post-mitochondrial testicular fractions, were used as an enzyme source. Testicular microsomal fractions were prepared as described in Andric et al. (2006), while characteristic of radioimmunoassay (RIA) for testosterone have been previously described in Andric et al. (2000a).

2.4. Leydig cell purification and incubation procedure

Isolation and purification of Leydig cells have been done according to Leckie et al. (1998) with some modifications. Briefly, adult male rats (around 90 days of age) were sacrificed by decapitation. Testes were quickly removed, decapsulated, placed in M199 containing 1.2 mg/ml collagenase, 1.5% BSA, 20 mM Hepes, 2.2 g/L sodium bicarbonate, 100 IU/ml penicillin and 100 μg/ml streptomycin, and incubated in a shaking water bath oscillating at 120 cycles/min at 34 °C for 15 min. The dissociated cells were filtered through Mesh No.100 (Sigma), and the resulting cell suspension was centrifuged twice at 160 × *g* for 5 min at room temperature. The crude suspension of interstitial cells was applied to a Percoll gradient consisting of four 2 ml-layers of Percoll with densities of 1.090, 1.080, 1.065, and 1.045 g/ml, and centrifuged at 500 × *g* for 28 min at room temperature. Fractions containing Leydig cells were collected from the 1.080/1.065 g/ml and 1.065/1.045 interface and cultured in DMEM/F12 medium supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin. The proportion of Leydig cells present in the culture was determined by staining for 3β- hydroxysteroid dehydrogenase (3βHSD) activity (Payne et al., 1980) and was typically more than 90%. The cells were plated on 96-well plate (50,000 cell/0.2 ml/well), allowed 3 h to attach and then exposed for 2 h to different doses of PCB126 or PCB153 in the presence of corresponding concentrations of P, or A^4 as substrates. PCB congeners and substrates were dissolved in M199 containing 0.1% BSA, 2.2 g/L sodium bicarbonate and antibiotics. The final DMSO concentration, which was used as a solvent for PCB congeners, was 0.1% and did not affect cell viability. After the end of treatment cell-free media was collected and stored at −20 °C prior to the measurement of T levels by RIA.

2.5. Enzyme assays

Denucleated fraction of interstitial testicular cells was obtained as previously described (Andric et al., 2003). The activities of superoxide dismutase (SOD), catalase, glutathione-*S*-transferase (GST), and glutathione peroxidase (GSH-Px) were estimated by procedures described in Andric et al. (2003).

The activities of CYP1A1 and CYP2B were measured as ethoxyresorufin-*O*-deethylase (EROD) and penthoxyresorufin-*O*-deethylase (PROD) activities in the microsomal fraction of testes and liver as previously described (Andric et al., 2006).

2.6. Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's multiple-range post hoc test was used for the analysis of the results from *in vivo* experiments. Mann–Whitney non-parametric test was used for the statistical analysis of the results from *in vitro* experiments, and $p < 0.05$ was considered as significant difference.

3. Results

3.1. Effect of PCB congeners on ex vivo androgenesis

Both PCB congeners had statistically significant effects on the conversion pathway from P to T, in which CYP17 and 17βHSD enzymes are involved, while combined application had no effect (Fig. 1). Higher dose of PCB126 decreased conversion of P to T, while lower dose of PCB153 had stimulating effect. In order to estimate which enzyme is affected in conversion pathway from P to T, we analyzed the activity of 17βHSD using A^4 as substrate. PCB126 decreased conversion of A^4 to T, in the same manner as conversion of P to T. However, PCB153 did

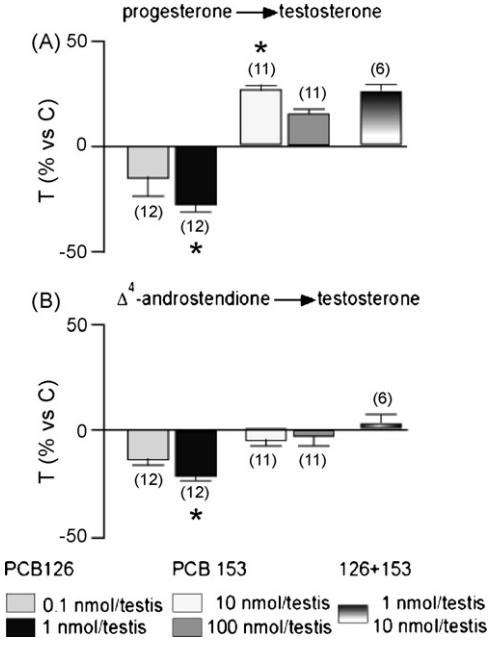

Fig. 1. Effects of *in vivo* application of PCB126 and PCB153 on the conversion of (A) progesterone and (B) Δ^4-androstenedione to testosterone in microsomal fraction of rat testis. The results are expressed as % of control minus 100%, and columns represent means ± S.E.M. values. Number in parenthesis represents number of animals per group. Significance: $^*p < 0.05$ vs. corresponding control.