Bull. Environm. Contam. Toxicol. 25, 65-68 (1980)

PCB Augments LH-Induced Progesterone Synthesis

G. B. Fuller, V. Knauf, W. Mueller*, and W. C. Hobson

*International Center of Environmental Safety, Albany Medical College, P.O. Box 1027, Holloman Air Force Base, New Mexico 88330, and *The Institute für Oekologische Chemie der Gesellschaft für Strahlen-und Umweltforschung mbH, Munich, Federal Republic of Germany*

A great deal of attention has been focused on various environmental contaminants and their toxic effects on mammalian systems. Important among the compounds studied are the organochlorine pesticides and the polychlorinated biphenyls (PCB). The accumulation and persistence of these contaminants in the biosphere has accentuated their public health significance and limited their widespread commercial utilization.

Changes in reproductive function have proven to be one of the more detrimental effects of chronic exposure to PCB or hexachlorobenzene (HCB). Reports in the literature have implicated potential target tissues such as the ovary (IATROPOULOS et al. 1976), steroid-hydroxylating enzymes in the liver (ORBERG & KIHLSTROM 1973, PLATONOW et al. 1972) and hypothalamus (GELLERT 1977).

The present experiment was designed to study the effect of acute in-vivo exposure to PCB (Clophen A-30) and HCB on LH-induced progesterone synthesis in-vitro. In order to obtain large amounts of lutein tissue relatively uniform in nature, the PMS-HCG primed immature rat model was utilized. This priming procedure, as described by PARLOW (1958), results in a pronounced capacity for progesterone synthesis by the excessive numbers of induced corpora.

METHODS

Twenty-one outbred albino female rats were utilized. Pregnant mares serum (PMS) was administered subcutaneously (sc) in saline to immature females on Day 29 (0 hour) at a dose level of 30 IU PMS. Sixty h following PMS 25 IU human chorionic gonadotropin (HCG) were injected sc in saline. On Day 34 (120 h) 7 animals each were randomly assigned to 3 groups as follows: sesame oil control, PCB-20 mg/kg BW (body weight) or HCB-20 mg/kg BW. On Day 36 animals were sacrificed by cervical dislocation and ovaries immediately removed and placed on ice. Tissues were pooled according to treatment groups, finely sliced and assigned to incubation vials in amounts ranging from 40-50 mg net wt. One-half of the incubation vials in each treatment group contained 10 IU ovine LH. The incubation was carried out in a Dubnoff metabolic shaker at 37° for 2 h as described by FULLER et al. (1978). Incubations were terminated by freezing on dry ice and vials were stored at -20° for subsequent analysis.

0007-4861/80/0025-0065 $01.00

At the time of progesterone assay tissue samples were thawed and homogenized in the incubation medium in 7-mL glass tissue grinders. Combined medium and tissue were extracted 3 times with 15 mL diethyl ether. Extracts were pooled, dried under vacuum, and re-dissolved in hexane. A 1% aliquot was taken for radioimmunoassay of progesterone as described by REYES et al. (1975).

Results were compared by an analysis of variance using the least significant difference test (STEELE & TORRIE 1960).

RESULTS

The results of four incubations are shown in Table 1. Since basal levels of progesterone in the unincubated tissue varied between incubations, data were normalized by expressing incubation values as a % of control tissue concentration of progesterone after exposure to ovine LH. The mean for incubated control tissue with LH, as shown in Table 1, is therefore 100.0.

Addition of LH to vials containing luteal slices from rats exposed to PCB resulted in approximately a 100% increase in progesterone synthesis above basal levels, whereas there was only a 31% increase with LH in control tissue. The increase in LH-induced progesterone synthesis in the corpora exposed to HCB was not statistically significant. In-vivo exposure of the luteinized ovaries to Clophen A-30 or HCB had no effect on in-vivo progesterone synthesis as there were no differences in pre-incubation levels of the steroid. Basal progesterone synthesis continued in all tissues as evidenced by a significant ($P<0.05$) increase above unincubated levels in all treatment groups.

Table 1. Effect of PCB or HCB on In-Vitro Progesterone Synthesis in Luteinized Rat Ovaries

Group	Animal Treatment	Tissue	Vials	Progesterone Content (Mean ± SE[a])
1	Control	Unincubated	10	41 ± 4
2		Incubated	14	70 ± 6[b]
3		Incubated with LH	16	100 ± 6[c]
4	PCB	Unincubated	13	55 ± 4
5		Incubated	17	93 ± 8[b]
6		Incubated with LH	18	180 ± 14[c,d]
7	HCB	Unincubated	7	39 ± 2
8		Incubated	12	99 ± 12[b]
9		Incubated with LH	14	110 ± 13

[a] Pooled from 4 incubations. Due to differing levels of basal progesterone synthesis between incubations, values were expressed as a % of mean concentration of LH-stimulated control tissue for that incubation.
[b] Significantly greater ($P<0.05$) than unincubated tissue.
[c] Significantly greater ($P<0.05$) than incubated tissue.
[d] Significantly greater ($P<0.05$) than LH-stimulated control tissue.

DISCUSSION

Although there are a few reports in the literature describing changes in reproductive function induced by PCB or HCB, the mechanism remains elusive. In this study PCB unexpectedly stimulated in-vitro progesterone biosynthesis. The lower chlorinated Aroclors (or Clophens) have been reported to be weak estrogens (BITMAN & CECIL 1970). As the PCB used in this study was 30% chlorine (up to 48% shows estrogenic activity) perhaps its estrogenicity might account for the enhanced LH-induced progesterone synthesis in these tissues. Whether the "estrogen" effect was a direct luteotropic stimulus or acted synergistically with LH cannot be determined from this study. That estrogens can be luteotropic in the rat was shown by TAKAYAMA & GREENWALD (1973) who reported that estradiol cyclopentylpropionate exerted a direct stimulatory action on the corpora lutea of a pregnant rat which was hysterectomized and hypophysectomized. However, the estrogen was luteotropic only after mid-pregnancy. Similar results in the rat were reported by GIBORI et al. (1977) but they found that early CL function also required estrogen but in combination with gonadotropins. In spite of functional similarities the luteotropic mechanism in PMS-induced corpora may differ from pregnancy corpora therefore the present data are merely suggestive. Nevertheless, since basal synthesis is comparable between controls and PCB-exposed tissues and the major treatment effect is induced by LH plus PCB exposure, this would indicate a synergistic relationship of PCB and LH.

There was no effect of HCB on LH-induced progesterone synthesis. Although the increase in basal synthesis appears to be greater than that in incubated control tissues it is difficult to attach biological significance to this result. HCB has been shown to reduce conception, litter size and lactation in rats but no specific mechanism for these effects has been found (GRANT et al. 1975).

These findings indicate that PCBs, but not HCB, act on ovarian tissue and support reports of an effect on reproductive function in rats. Whether the reproductive consequences of exposing rats to the lower chlorinated PCBs can be attributed to their estrogenicity remains to be determined.

Acknowledgements. This was supported in part by the Federal Department of Research and Technology of the Federal Republic of Germany, under a coordinated research program entitled "Ecologic-Toxicologic Effects of Foreign Compounds in Non-Human Primates and Other Laboratory Animals". Ovine LH was a gift from the NIAMDD by L. Reichert of Emory University. We also acknowledge B. M. Markaverich for his suggestions.

REFERENCES

BITMAN, J., and H. C. CECIL: J. Agr. Food Chem. 18, 1108 (1970).
FULLER, G., B. M. MARKAVERICH, and W. C. HOBSON: Acta Endo. 87, 164 (1978).

GELLERT, R. J.: Environ. Res. 16, 123 (1975).
GIBORI, G., E. ANTCZAK, E., and I. ROTHCHILD: Endocrinology 100, 1483 (1977).
GRANT, D. L., G. V. HATINA, and W. E. J. PHILLIPS: Toxicol. Appl. Pharm. 33, 167 (Abst.)
IATROPOULOS, M. J., W. HOBSON, V. KNAUF and H. P. ADAMS: Toxicol. Appl. Pharm. 37, 433 (1976).
ORBERG, J. and J. E. KIHLSTROM: Environ. Res. 6, 176 (1973).
PARLOW, A. F.: Fed. Proc. 17, 402 (1958).
PLATONOW, N. S., R. M. LIPTRAP, and H. D. GEISSINGER: Bull. Environ. Contam. Toxicol. 7, 358 (1972).
REYES, F. I., J. S. D. WINTER, C. FAIMAN, and W. C. HOBSON: Endocrinology 96, 1447 (1975).
STEELE, R. G. D., and J. H. TORRIE: Principles and Procedures of Statistics:, 426 pp. New York, Mc-Graw Hill 1960.
TAKAYAMA, M., and G. S. GREENWALD: Endocrinology 92, 1405 (1973).