Bull. Environm. Contam. Toxicol. 20, 199-205 (1978)

Hepatic and Renal Microsomal Electron Transport Reactions in Endrin Treated Female Guinea Pigs

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Halogenated hydrocarbon insecticides and a vast number of chemicals can induce nonspecifically drug metabolizing enzymes in animals (HART et al.,1963; HART and FOUTS,1963, 1965; WELCH et al.,1967; GILLETT,1968; BUNYAN and PAGE, 1973). Administration of endrin was shown to cause decrease in sleeping time due to hexobarbital (HART and FOUTS,1963),thiopental and pentobarbital (KACHOLE and PAWAR,1977) in rats. Recently HARTGROVE et al.(1974) observed that endrin causes no increase in mixed function oxidase activity using ethylmorphine as model substrate. The toxicity of endrin has been shown to be greater in female guinea pigs than in males (TREON and CLEVELAND,1955). Data pertaining to the effects of endrin on microsomal mixed function oxidase system in guinea pigs is not available. Hence the present experiments were designed to study the effects of endrin on the levels of electron transport components, aminopyrine N-demethylation and lipid peroxidation in adult female guinea pigs.

MATERIALS AND METHODS

Adult female guinea pigs weighing 600-650 g, obtained from Hindustan Antibiotics, Pune, were caged separately into wire bottom cages in an air conditioned room maintained at 25°C. The animals were fed on standard laboratory diet and tap water ad libitum for two to three weeks prior to the utilization for experiments. Animals were then divided into two groups of six animals each. One group of animals was injected with endrin (3 mg/kg in safola oil) intraperitoneally daily in the morning between 8.00 to 9.00 a.m. for three successive days. The control group received an equivalent amount of the vehicle oil.

Twentyfour hours after the last injection, the animals were killed by cervical dislocation. Livers and kidneys were removed after perfusion with 0.9 % icecold saline and microsomes were prepared according to the proceedure reported earlier(PAWAR and MAKHIJA,1975). The microsomal protein was estimated by biuret method (GORNALL et al.,1949) using crystalline bovine serum albumin as the standard.

Aminopyrine N-demethylase activity was measured according to the proceedure already reported (PATEL and PAWAR,1974), using both TPNH and DPNH as electron donors. The effect of addition of cyanide and ascorbate in the reaction mixture was also investigated.

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0007-4861/78/0020-0199 $01.40

Microsomal lipid peroxidation was assayed as described by ERNSTER and NORDENBRAND (1967) using TPNH as well as DPNH as electron donors. The effect of ascorbate and cyanide addition on in vitro lipid peroxidation was also investigated. The malonaldehyde formed was estimated by the thiobarbituric acid reaction (BERNHEIM et al.,1948).

Microsomal electron transport components such as cytochrome P 450, cytochrome b5 and total heme were determined on a Hitachi (Model 124) recording spectrophotometer as recently reported (MAKHIJA and PAWAR,1977).

RESULTS

Organ weights and microsomal protein content:
Endrin treatment caused a significant increase in liver weight and decrease in hepatic microsomal protein content. However, kidney weight and renal microsomal protein content were not affected.

Electron transport components in liver microsomes:
Increase in the hepatic cytochrome b5 content and cytochrome c reductase activity was noticed in endrin treated guinea pigs. However, cytochrome P 450 and total heme were found to be decreased significantly.

Hepatic aminopyrine N-demethylase activity:
Endrin administration caused a decrease and increase in the rates of aminopyrine N-demethylation in presence of TPNH and DPNH respectively. When TPNH was used in combination with DPNH,it was observed that the rate of in vitro metabolism of aminopyrine remains unaltered in comparison with TPNH linked system and in comparison with the DPNH linked system, it was found to be enhanced in case of control animals. However, a marked increase in the enzyme activity is noticed in case of endrin treated animals. The percent increase was 50 and 60 respectively in TPNH and DPNH linked drug metabolism. In vitro addition of cyanide caused an increase in DPNH linked rate of aminopyrine N-demethylation in endrin treated animals. Addition of ascorbate to DPNH linked system increased the rate of demethylation by three fold in control animals and four fold in endrin treated animals respectively. However, stimulatory effect of ascorbate was reduced significantly when cyanide was added together with ascorbate.

Renal aminopyrine N-demethylase activity:
The activity of renal aminopyrine N-demethylase was significantly lower than that of hepatic. A marginal increase in the enzyme activity in TPNH linked system and about two fold increase in DPNH mediated system was observed due to endrin treatment. Combination of DPNH and TPNH did not enhance the enzyme activity in case of control guinea pigs. However, three fold increase was noticed in case of endrin treated animals. In vitro effect of cyanide and ascorbate on DPNH linked demethylation was similar to that in the hepatic system.

TABLE I
EFFECT OF ENDRIN ON FEMALE GUINEA PIG HEPATIC MIXED FUNCTION OXIDASE SYSTEM.

PARAMETER	GROUP	
	CONTROL	ENDRIN TREATED
Relative liver weight(% b.wt)	3.56± 0.12	4.02± 0.20
Microsomal protein (mg/g liver)	45.20±0.14	35.79±0.09
Cytochrome b5*	0.135±0.008	0.260±0.010
Cytochrome P 450*	0.330±0.005	0.284±0.012
Total Heme*	0.600±0.018	0.560±0.010
Cytochrome c reductase**	24.0 ± 1.4	42.6± 2.6
Aminopyrine N-demethylase activity (n moles of formaldehyde formed/min/mg protein)		
TPNH	16.00± 0.20	14.00± 0.15
DPNH	11.25± 0.10	11.67± 0.10
TPNH+DPNH	16.25± 0.25	21.00± 0.20
DPNH+KCN	11.25± 0.15	16.87± 0.25
DPNH+Ascorbate	33.62± 0.30	42.50± 0.35
DPNH+KCN+ Ascorbate	29.00± 0.20	35.12± 0.30
Lipid peroxidation (n moles of malonaldehyde formed/min/mg protein)		
TPNH	5.00± 0.20	11.80± 0.30
DPNH	11.82± 0.25	10.88± 0.15
TPNH+DPNH	5.90± 0.10	6.36± 0.10
DPNH+KCN	4.32± 0.05	4.85± 0.10
Ascorbate	8.85± 0.25	12.35± 0.20
Non Enzymatic	2.70± 0.10	3.14± 0.05

* n moles / mg microsomal protein .
** n moles of cytochrome c reduced/min/mg protein .
Each value represents mean of six animals .

TABLE II
EFFECT OF ENDRIN ON FEMALE GUINEA PIG RENAL AMINOPYRINE N-DEMETHYLATION AND LIPID PEROXIDATION.

PARAMETER	GROUP	
	CONTROL	ENDRIN TREATED
Relative kidney weight(% b.wt.)	0.771± 0.008	0.840± 0.012
Kidney microsomal protein mg/g tissue	27.50± 0.16	27.89± 0.20
Aminopyrine N- demethylation (n moles of formaldehyde formed/min/mg protein)		
TPNH	10.80± 0.10	11.70* 0.14
DPNH	6.12± 0.12	11.67± 0.07
TPNH+DPNH	6.08* 0.09	19.62± 0.12
DPNH+KCN	6.20± 0.12	16.87± 0.10
DPNH+Ascorbate	29.00± 0.27	32.87± 0.18
DPNH+KCN+ Ascorbate	36.02± 0.22	29.75± 0.20
Lipid peroxidation (n moles of malonaldehyde formed/min/mg protein)		
TPNH	18.1 ± 0.1	20.8 ± 0.2
DPNH	10.3 ± 0.1	17.9 ± 0.2
TPNH+DPNH	15.3 ± 0.2	16.8 ± 0.1
DPNH+KCN	16.0 ± 0.2	19.8 ± 0.2
Ascorbate	19.7 * 0.2	24.6 ± 0.2
Non enzymatic	2.8 ± 0.1	2.3 ± 0.1

Each value represents mean of six animals.

The percentage of induction due to cyanide was very high. Addition of cyanide and ascorbate to the reaction mixture in combination influenced similar effects in controls as well as endrin treated animals.

Hepatic lipid peroxidation:
The levels of lipid peroxides formed in DPNH induced system were higher than in the TPNH and ascorbate promoted system in untreated animals. Addition of 1 mM KCN to the DPNH linked lipid peroxidation was found to have an inhibitory effect. Endrin treatment resulted in a slight decrease in DPNH induced lipid peroxidation. However, all other combinations of electron donors TPNH,TPNH+DPNH,ascorbate etc.resulted in a significant increase in lipid peroxidation in hepatic microsomes.

Renal lipid peroxidation:
Ascorbate induced lipid peroxidation was found to be the highest in control as well as treated female guinea pigs, renal microsomes. TPNH linked lipid peroxidation was slightly lower than ascorbate induced, whereas the DPNH mediated lipid peroxidation was the least of all in untreated animals. In contrast to liver, KCN was found to enhance the renal DPNH promoted lipid peroxidation. Endrin treatment caused a gross increase in lipid peroxidation using various electron donors.

DISCUSSION

CONNEY et al.(1967) and ESTABROOK et al.(1971) observed a marked increase in the rate of drug metabolism due to the synergistic effect of TPNH and DPNH. On the basis of later studies, COHEN and ESTABROOK (1971 a,b,c), HILDEBRAND and ESTABROOK (1971), and CORREIA and MANNERING (1973) have postulated the involvement of cytochrome b5 in biotransformation of various drugs. WEST et al.(1974) have recently postulated the obligatory requirement of cytochrome b5 in the DPNH mediated benzpyrene hydroxylation via cytochrome P 450. In vitro effect of cyanide on DPNH linked metabolism of aminopyrine via cytochrome b5 system suggests insensitiveness of DPNH mediated system to cyanide.This is supported by similar observations of SHIGAMATSU et al. (1976) in rabbits. In vitro effect of ascorbate on DPNH mediated reactions indicate the involvement of ascorbate dependent DPNH oxidase system. The metabolism of aminopyrine via this system has been reported in guinea pigs (ZANNONI et al.,1972).

Our present studies indicate a decrease in TPNH linked aminopyrine N-demethylation during endrin intoxication, which is due to the decreased levels of cytochrome P 450. This finding is supported by the recent report of HAYES et al.(1974), who observed a considerable decrease in cytochrome P 450 due to in vitro addition of endrin

The present studies also indicate that endrin treatment caused an increase in the in vitro metabolism of aminopyrine by DPNH mediated system with the parallel increase in the cytochrome b5 and cytochrome c reductase and that this system is not affected by cyanide. These studies suggest that the induction of cytochrome b5 facilitates the flow of electrons in DPNH mediated aminopyrine N-demethylation . The observed increase in lipid peroxidation may be due to the increased levels of total lipids and phospholipids. Enhanced lipid peroxidation and its inhibition by cyanide during endrin intoxication also indicate the involvement of cytochrome b5 as a major factor in governing the process of mixed function oxidase system.

ACKNOWLEDGEMENTS

This work is partially supported by Council of Scientific and Industrial Research, New Delhi,India, by awarding Junior Research Fellowship to M.S.K. Authors acknowledge the help of Prof. R. W. Estabrook, Department of Biochemistry, Health Science Centre, University of Texas, Dallas, Texas, U.S.A., during the work. The authors also wish to thank Dr. S.J.Makhija for her help in all stages of the work. Endrin was a kind donation of Sandoz (India) Limited, Bombay.

REFERENCES

BERNHEIM,F.,BERNHEIM,M.L.C. and WILBUR,L.:J.Biol.Chem.174, 247 (1948).
BUNYAN,P.J. and PAGE,J.M.J.:Chem.-Biol.Interac.6,249(1973).
COHEN,B.S. and ESTABROOK,R.W.:Arch.Biochem.Biophys. 143,37(1971).
————————:——ibid,143,46(1971)
————————:——ibid,143,54(1971)
CONNEY,A.H., WELCH,R.M., KUNZTMAN,R. and BURNS,J.J.: Clin.Pharmacol.Ther. 8.2(1967).
CORREIA,M.A. and MANNERING,G.J.: Mol.Pharmacol. 9,455(1973).
ERNSTER,L. and NORDENBRAND,K.: Methods in Enzymol. 10,574(1967).
ESTABROOK,R.W., FRANKLIN,M.R., COHEN,B., SHIGAMATSU,A., and HILDEBRANDT,A.G.: Metabolism. 20,186(1971).
GILLETT,G.W.: J.Agric.Food Chem. 16,295(1968).
GORNALL,A.G., BARDAWILL,C.J. and DAVID,M.M.: J.Biol.Chem. 177,751(1949).
HART.L.G. and FOUTS,J.R.: Proc.Soc.Exp.Biol.Med. 114,388 (1963).
HART.J.G. and FOUTS,J.R.: Arch.Pharmakol. Exp.Pathol. 249,486(1965).
HART,L.G., SCHULTICE,R.W. and FOUTS,J.R.: Toxicol.Appl. Pharmacol. 6,371(1963).
HARTGROVE,R.W.Jr., HUNDLEY,S.G. and WEBB,R.E.: Toxicol. Appl.Pharmacol. 29,156(1974).
HAYES,J.R., HARTGROVE,R.W., HUNDLEY,S.G., CAMPBELL,T.C., and WEBB,R.E.: Toxicol.Appl.Pharmacol. 29,75(1974).

HILDEBRANDT,A.G. and ESTABROOK,R.W.: Arch.Biochem.Biophys. 143,66(1971).
KACHOLE,M.S. and PAWAR,S.S.: Ind.J.Biochem.Biophys. 14,45(1977)Suppl.
MAKHIJA,S.J. and PAWAR,S.S.: Ind.J.Exper.Biol. 15, (1977).
PATEL,J.M. and PAWAR,S.S.: Biochem.Pharmacol. 23,1467(1974).
PAWAR,S.S. and MAKHIJA,S.J.: Bull.Environ.Contam.Toxicol. 14,197(1975).
SHIGAMATSU,H., YAMANO,S. and YOSHIMURA,H.: Arch.Biochem. Biophys. 173,178(1976).
TREON,J.F. and CLEVELAND,F.P.: J.Agric.Food.Chem. 3,402(1955).
WELCH,R.M., LEVIN,W. and CONNEY,A.H.: J.Pharmacol.Exper. Ther. 155,167(1967).
WEST,S.B., LEVIN,W., RYAN,D., VORE,M. and LU.A.Y.H.: Biochem.Biophys.Res.Commun. 58,516(1974).
ZANNONI,V.G., FLYNN,E.J. and LYNCH,M.: Biochem.Pharmacol. 21,1377(1972).