Biochemical Pharmacology, Vol. 32, No. 22, pp. 3479–3480, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00
Pergamon Press Ltd.

SHORT COMMUNICATIONS

The effects of phenobarbital, 3-methylcholanthrene and benzo(*a*)pyrene on the hydrolysis of xenobiotics in the rat

(*Received* 18 *February* 1983; *accepted* 7 *June* 1983)

It has become increasingly apparent in the last couple of years that non-specific microsomal carboxylesterases/amidases or B-esterases (EC 3.1.1.1), which exist in heterogeneous multiple forms in mammalian tissues, play an important role in the hydrolytic metabolism of a large number of xenobiotics containing a carboxylester, thioester or arylamide linkage, and thus play an important role in drug metabolism, carcinogenesis and detoxification or potentiation of toxic effects of many noxious chemicals present in our environment [1–5]. Recently, these hydrolases have been shown to be potential sites of metabolic interactions among xenobiotics [5–7].

Phenobarbital (PB) and carcinogenic polycyclic aromatic hydrocarbons (PAH) such as 3,4-benzo(*a*)pyrene (BP) and 3-methylcholanthrene (3-MC) have been extensively employed as microsomal enzyme inducers to resolve and characterize heterogeneous forms of cyt. P-450 dependent mixed function oxidases [8] and UDP-glucuronyltransferases [9]. However, our knowledge of the responses of carboxylesterases/amidases to xenobiotics is fragmentary and leads to divergent conclusions. PB administration to rats has previously been shown to enhance the hepatic metabolism of procaine and isocarboxazid [10, 11] and *p*-nitrophenylacetate [12] whereas reduce the rate of hydrolysis of aspirin to salicylic acid [13]. Another report, on the contrary, has shown a slight inhibition of procaine hydrolysis in liver microsomes from PB treated rats [14]. By crossed immunoelectrophoresis of hepatic microsomal carboxylesterases from PB-induced rats, it was shown that an acetanilid specific carboxylesterase/amidase was selectively induced by short-term PB treatment while other esterase-active antigens remained unaffected [15]. Elucidation of the dose-dependent kinetics of induction of α-naphthylacetate esterase in rat liver by PB and DDT led to infer that a critical high dosage must be administered before induction of the enzyme is elicited [16]. The enzyme activity remained unchanged after intraperitoneal PB administration at a dose of 40 mg/kg for five consecutive days but remarkably when PB was given at a dose of 120 mg/kg under similar experimental conditions. Likewise, the induction of esterase by daily intraperitoneal DDT treatment for 4 days in low-dosage group (50 mg/kg) was 55%, which was remarkably less compared to 195% observed in high-dosage group (150 mg/kg). A study undertaken recently to investigate the induction kinetics of a microsomal carboxylesterase hydrolysing NPA in rat liver reported a significant increase in the enzyme activity only after 4 days of daily intraperitoneal PB (100 mg/kg) administration which reached a maximum after 6 days treatment [12]. Much less information is available on the influence of 3-MC, BP or other PAH on the hydrolytic metabolism of xenobiotics catalysed by different forms of B-esterases.

The current study was designed to provide an insight into the possible changes occurring in the activities of esterases/amidases catalysing hydrolytic metabolism of acetylsalicylic acid (ASA), procaine, *p*-nitrophenylacetate (NPA), acetanilid and 2-acetylaminofluorene (AAF), a carcinogen, in liver, lung, intestine, kidney and brain of rats exposed to PB, 3-MC and BP. The activity of cholinesterase was also compared under similar experimental conditions.

Materials and methods

Chemicals. 2-Acetylaminofluorene, 3,4-benzo(*a*)pyrene, 3-methylcholanthrene and bovine serum albumin were obtained from Sigma Chemical Co. St. Louis, MO, U.S.A. Butyrylcholine iodide and *p*-nitrophenylacetate were acquired from Koch-Light Lab., Colnbrook Bucks, England, and Sisco Research Lab. Pvt. Ltd., India respectively. Phenobarbital was a gift from Indian Drugs and Pharmaceuticals Ltd. Trimethamine (Tris) and procaine hydrochloride were purchased from BDH-Glaxo Lab. (India) Ltd. and May and Baker Ltd. (India) respectively. All other chemicals were of analytical grade.

Female Albino rats (100–150 g) kept on *ad lib.* diet were treated intraperitoneally for six consecutive days with high dosage of PB, 3-MC or BP (80 mg/kg per day) in peanut oil. The same amount of peanut oil administered to controls did not affect the activities of esterases/amidases. The animals, unless otherwise stated, were killed 18–20 hr after the last dose by decapitation. Tissues were removed and washed well with 1.15% KCl. To remove blood, livers were cut into small pieces and thoroughly washed with ice-cold 1.15% KCl before homogenization. Small intestine was used as the source of intestinal hydrolases. Tissues were homogenized in chilled 1.15% KCl with the help of Potter-Elvehjem homogenizer and liver microsomes were prepared according to established procedures using MSE 75 ultracentrifuge [17]. The activities of ASA esterase I (active at pH 5.5), ASA esterase II (active at pH 7.4), procaine esterase, NPA esterase, acetanilid *N*-deacetylase and cholinesterase in liver microsomes and homogenates of lung, intestine, kidney and brain were determined spectrophotometrically by the methods previously described [18]. The rate of hydrolysis of AAF was measured as described earlier [2]. The reaction mixture (1 ml) containing Tris–HCl buffer (0.1 M, pH 9.0), 1×10^{-3}M 2-AAF and tissue homogenate or liver microsomes was incubated at 37° and the product aminofluorene was estimated spectrophotometrically. Protein was determined by the method of Lowry *et al.* [19].

Results and discussion

The results of the current investigation revealed marked stimulation of hepatic microsomal carboxylesterases/amidases necessary for the hydrolytic metabolism of ASA, procaine, NPA, acetanilid and AAF in rats pretreated with PB (80 mg/kg per day) for six consecutive days (Table 1). Under such conditions, the activity of cholinesterase remained unchanged. There was 2.5-, 2.4-, 2.0-, 1.9-, 1.7- and 2.1-fold increase in the activity of ASA esterase I, ASA esterase II, procaine esterase, NPA esterase, acetanilid *N*-deacetylase and AAF *N*-deacetylase respectively. These increases were the result of enzyme induction since the activities of the hepatic carboxylesterases and amidases were not increased 1 hr after an oral dose of PB (80 mg/kg) or by the addition of PB (1 mM) to *in vitro* incubations.

Table 1. Effect of phenobarbital, 3-methylcholanthrene and benzo(*a*)pyrene on hepatic microsomal esterases/amidases

Esterases/Amidases	Enzyme activity* (nmoles product formed or substrate disappeared/min/mg protein)			
	Control	PB	3-MC	BP
ASA esterase I	87 ± 3.2	222 ± 9.8†	91 ± 4.3	82 ± 2.8
ASA esterase II	75 ± 2.1	178 ± 6.2†	80 ± 3.3	69 ± 3.0
Procaine esterase	1.48 ± 0.09	3.00 ± 0.21†	1.58 ± 0.11	1.42 ± 0.08
NPA esterase	2415 ± 149	4540 ± 410†	2583 ± 111	2490 ± 132
Acetanilid *N*-deacetylase	22.8 ± 1.0	38.7 ± 2.8†	20.3 ± 0.61	23.5 ± 0.82
AAF *N*-deacetylase‡	5.81 ± 0.31	12.0 ± 0.62†	6.21 ± 0.22	5.14 ± 0.28
Cholinesterase	3.75 ± 0.28	4.11 ± 0.42	3.59 ± 0.21	4.21 ± 0.31

* Values are the mean ± S.E. calculated from 7–8 rats. The rats were administered with PB, 3-MC or BP intraperitoneally at a dose of 80 mg/kg per day for six consecutive days.

† Significantly different from control, $P < 0.001$.

‡ One unit of AAF *N*-deacetylase represents the amount of enzyme causing an increase of 0.001 optical density in 1 min under the experimental conditions described.

The activities of esterases/amidases in lung, intestine, kidney and brain were not modified after repeated PB administration. This observation agrees with other studies which have demonstrated inability of PB to induce α-naphthylacetate esterase [16], acetanilid *N*-deacetylase [15] and NPA esterase [12] in rat. Likewise, it is reported from our laboratory that chronic oral tobacco [18] and methaqualone [20] consumption selectively induced hepatic B-esterases while the activities of renal and neuronal enzymes remained unchanged.

Interestingly, daily intraperitoneal administration of 3-MC or BP at a dose of 80 mg/kg for 6 days did not elicit a change in the activities of ASA esterase I, ASA esterase II, procaine esterase, NPA esterase, acetanilid *N*-deacetylase, AAF *N*-deacetylase and cholinesterase in liver (Table 1) and other tissues of rat. Earlier communications have demonstrated insensitiveness of hepatic microsomal isocarboxazid amidase [10] and acetanilid *N*-deacetylase [15] to 3-MC treatment but the dosage of the carcinogen was comparatively low (25 mg/kg per day, for 3–4 days).

In conclusion, the present study confirms that PB is a potent non-specific inducer of hepatic microsomal esterases and amidases. However, no such induction was detected in other tissues or when high doses of polycyclic aromatic hydrocarbons were employed as potential inducing agents.

Acknowledgements—We thank Professor K. P. Bhargava for encouragement. Thanks are due to Indian Drugs and Pharmaceuticals Ltd. for generous gift of phenobarbital. Grateful acknowledgement is made to the Indian Council of Medical Research for providing a Research Associateship to S. Kaur.

Jawahar Lal Nehru Laboratory of Molecular Biology
Department of Pharmacology and Therapeutics
King George's Medical College
Lucknow 226003
India

SWARAJ KAUR
BASHEER ALI*

* Correspondence should be addressed to Dr Basheer Ali, Visiting Scientist, Department of Physiology, School of Medicine, University of North Dakota, Grand Forks, North Dakota 58202, U.S.A.

and
Departments of Physiology and Surgery
University of North Dakota
School of Medicine
Grand Forks, North Dakota 58202
U.S.A.

REFERENCES

1. K. Krisch, in *The Enzymes* (Ed. P. D. Boyer), Vol. 5, p. 43. Academic Press, New York (1971).
2. M. Jarvinen, R. S. S. Santti and V. K. Hopsu-Havu, *Biochem. Pharmac.* **20**, 2971 (1971).
3. B. N. La Du and H. Snady, in *Concepts in Biochemical Pharmacology, Handbook of Experimental Pharmacology* (Eds. B. B. Brodie and J. R. Gillette), Vol. 2, p. 477. Springer, Berlin (1971).
4. R. Mentlein, S. Heiland and E. Heymann, *Archs Biochem. Biophys.* **200**, 547 (1980).
5. E. H. Silver and S. D. Murphy, *Toxic. Appl. Pharmac.* **57**, 208 (1981).
6. T. Satoh and K. Moroi, *Jap. J. Pharmac.* **27**, 233 (1977).
7. G. P. Carlson, J. D. Dziezak and K. M. Johnson, *Res. Commun. Chem. Path. Pharmac.* **25**, 181 (1979).
8. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
9. K. W. Bock, D. Josting, W. Lilienblum and H. Pfeil, *Eur. J. Biochem.* **98**, 19 (1979).
10. T. Satoh and K. Moroi, *Life Sci.* **12**, 169 (1973).
11. K. Moroi and T. Satoh, *Biochem. Pharmac.* **24**, 1436 (1975).
12. U. Nousiainen and O. Hännien, *Acta Pharmac. Toxic.* **49**, 77 (1981).
13. J. F. Howes and W. H. Hunter, *J. Pharm. Pharmac.* **20**, 107 (1968).
14. H. Remmer, *Proc. Europ. Soc. for Study of Drug Toxicity* **4**, 57 (1964).
15. M. Raftell, K. Berzins and F. Blomberg, *Archs Biochem. Biophys.* **181**, 534 (1977).
16. W. S. Schwark and D. J. Ecobichon, *Can. J. Physiol. Pharmac.* **46**, 207 (1967).
17. S. Kaur and B. Ali, *Res. Commun. Chem. Path. Pharmac.* **38**, 343 (1982).
18. B. Ali and S. Kaur, *Biochem. Pharmac.* **31**, 3683 (1982).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. S. Kaur and B. Ali, *Toxic. Appl. Pharmac.* in Press (1983).