*Biochemical Pharmacology*, Vol. 31, No. 22, pp. 3683–3684, 1982.
Printed in Great Britain.

0006-2952/82/223683-02 $03.00/0


# SHORT COMMUNICATIONS

## Influence of oral tobacco and nicotine consumption on the hydrolytic metabolism of xenobiotics

(*Received* 1 *February* 1982; *accepted* 21 *April* 1982)

A number of studies carried out in the past have shown that esterases/amidases, a group of hydrolases, play an important role in the metabolism of drugs [1–3], carcinogens [4] and chemicals used in agriculture [1] and industry [5]. It is also demonstrated that a large number of metabolic interactions with xenobiotics of the ester or amide type can be attributed to an alteration in the activity of these microsomal hydrolases [5–7]. We have recently reported an induction of hepatic microsomal mixed function oxidases (MFO) responsible for the metabolism of drug and carcinogen in rats receiving tobacco or nicotine orally for prolonged periods [8, 9]. The purpose of the present investigation was to evaluate the effect of chronic oral tobacco and nicotine consumption on the hydrolytic metabolism of aspirin (acetylsalicylic acid or ASA), procaine, *p*-nitrophenylacetate (NPA), acetanilid and butyrylcholine in the liver, kidney and brain of rat. The responses of tissue esterases/amidases to these xenobiotics of abuse were also examined in the rats given acute treatment.

*Chemicals.* Butyrylcholine iodide and bovine serum albumin were obtained from Koch-Light Lab. Ltd. (Colnbrook, Bucks, U.K.) and Sigma Chemical Co. (St. Louis, MO), respectively. *p*-Nitrophenylacetate was supplied by Sisco Research Lab. Pvt. Ltd. (India). Trimethamine (Tris) and procaine hydrochloride were obtained from BDH-Glaxo Lab. (India) Ltd. and May & Baker Ltd. (India). All other chemicals were of analytical grade and obtained from commercial sources.

Female Albino rats (150–200 g) were administered orally commercially available sun-cured tobacco leaves (250 mg/kg) or nicotine (10 mg/kg) twice daily for 1, 7 and 25 days under the experimental conditions described earlier [8]. The animals were killed 18–20 hr after the last dose of tobacco or nicotine and homogenates of liver, kidney and brain prepared in 1.15% KCl [8] were assessed for their ability to hydrolyse the substrates. Under the standard experimental conditions, the rate of reactions was linear with respect to time and enzyme protein.

*Assay of ASA esterases.* According to our unpublished results (Basheer Ali and Swaraj Kaur, manuscript in preparation), the hydrolysis of aspirin in liver and other tissues of rat was found to occur at two pH levels, i.e. 5.5 and 7.4, and the two enzymes/isozymes, designated as ASA esterase I and II respectively, could be distinguished from each other on the basis of *in vitro* response observed during their interaction with metals and inhibitors/activators. The rate of ASA hydrolysis was followed by estimating the product salicylic acid. The reaction mixture (1 ml) containing acetate buffer (0.2 M, pH 5.5) or Tris–HCl buffer (0.2 M, pH 7.4), 0.008 M ASA and tissue homogenate was incubated at 37°. The reaction was terminated by addition of ferric–mercuric reagent to determine salicylic acid [10].

*Assay of procaine esterase and butyrylcholine esterase.* The activities of procaine esterase and butyrylcholine esterase were assayed by following the disappearance of procaine and butyrylcholine, respectively, spectrophotometrically [11].

*Assay of NPA esterase.* The determination of unspecific carboxylesterase activity was based on the spectrophotometric estimation of *p*-nitrophenol, a hydrolytic product of *p*-nitrophenylacetate, which gives a yellow colour in alkaline medium. The reaction mixture (1 ml) containing Tris–HCl buffer (0.1 M, pH 7.4), 0.003 M NPA and tissue homogenate was incubated at 30° for 10 min. The reaction was terminated with TCA and a suitable aliquot of the supernatant thus obtained was made alkaline with Tris–HCl buffer (1 M, pH 7.4) to read the yellow colour at 415 nm.

*Assay of acetanilid* N-*deacetylase.* Spectrophotometric estimation of aniline formed during hydrolysis of acetanilid was taken as an index of acetanilid *N*-deacetylase activity of tissues. The reaction mixture (1 ml) consisted of Tris–HCl buffer (0.2 M, pH 8.5), 0.01 M acetanilid and tissue homogenate. After the desired incubation period, the reaction was terminated by addition of TCA and aniline was determined in the supernatant [12].

*Protein determination.* Protein was determined by the method described previously [13].

*Results and discussion.* The present investigation revealed marked stimulation of the hydrolytic metabolism of ASA and acetanilid in the liver following chronic exposure of rats to oral tobacco (250 mg/kg) twice daily for 25 days (Table 1). The per cent increase in the activity of ASA esterase I and II, and acetanilid *N*-deacetylase was 52, 36 and 141 respectively. Such tobacco treatment was devoid of any effect on the hydrolysis of procaine, NPA and butyrylcholine. The stimulation kinetics of ASA esterase I and II and acetanilid *N*-deacetylase with respect to time was elucidated by investigating the enzyme activities after 1 and 7 days of tobacco treatment. The enzyme activities were not influenced after 1 day of tobacco treatment. Likewise, a single oral dose of tobacco also failed to alter the hepatic metabolism of the xenobiotics. However, tobacco treatment for 7 consecutive days caused 1.32-, 1.23- and 2.0-fold enhancement in the activities of ASA esterase I and II and acetanilid *N*-deacetylase respectively, which is significantly less compared to that observed after 25 days of tobacco treatment. These observations suggest that the process of enzyme stimulation is slow and therefore repeated dosage for a prolonged period is required to accelerate the rate of aspirin and acetanilid metabolism, presumably as a result of slow increase in the *de novo* synthesis of ASA esterases and acetanilid *N*-deacetylase. Furthermore, since the activities of procaine esterase, NPA esterase and butyrylcholine esterase were not affected, it appears that tobacco is a specific inducer of hepatic esterases/amidases. The results of the current study suggest that the enzyme or isozyme catalysing the hydrolysis of acetanilid is probably not genetically linked with those necessary for the metabolism of procaine, NPA and butyrylcholine. This finding does not support the earlier view suggesting existence of a single microsomal carboxylesterase/amidase of B-type (EC 3.1.1.1) for the metabolism of procaine and amide-type drugs such as isocarboxazid and acetanilid in the liver from monkey [14] and rat [15, 16], since it was not possible to dissociate the enzymes involved in the hydrolysis of these substrates.

Interestingly, oral tobacco treatment to rats was unable

Table 1. Effect of oral tobacco administration on hepatic esterases/amidases in rat

| Esterase/amidase | Control | Enzyme activity (units/mg protein) Treated 1 day | 7 days | 25 days |
|---|---|---|---|---|
| ASA esterase I | 230 ± 11 | 205 ± 12 | 305 ± 18* | 351 ± 17* |
| ASA esterase II | 198 ± 9 | 179 ± 7 | 245 ± 6* | 270 ± 18* |
| Procaine esterase | 3.58 ± 0.12 | 4.02 ± 0.22 | 3.25 ± 0.21 | 3.72 ± 0.11 |
| NPA esterase | 7152 ± 221 | 8053 ± 526 | 7632 ± 331 | 6857 ± 412 |
| Acetanilid *N*-deacetylase | 52.1 ± 1.8 | 58.3 ± 2.4 | 104 ± 4* | 126 ± 6.8* |
| Butyrylcholine esterase | 15.5 ± 1.2 | 14.8 ± 1.0 | 13.2 ± 0.91 | 17.2 ± 0.8 |

Values are means ± S.E. with separate homogenates of liver obtained from 8–10 rats. One unit of esterase/amidase activity is expressed as the amount of enzyme causing disappearance or appearance of 0.1 nmole of substrate or product respectively under the experimental conditions described.
* $P < 0.01$.

to alter the activities of esterases/amidases necessary for the metabolism of ASA, procaine, NPA, acetanilid and butyrylcholine in kidney and brain throughout the course of study. Similar findings have been reported previously, where phenobarbital [17, 18] and DDT [17] induced hepatic carboxylesterases/amidases but did not modify the enzyme activity of extrahepatic tissues. It seems that extrahepatic enzymes are probably non-inducible in nature.

Oral administration of nicotine (10 mg/kg, twice a day), equivalent to that present in 250 mg tobacco, did not elicit any change in the rate of hydrolytic metabolism of the xenobiotics by hepatic or extrahepatic esterases/amidases. It suggests that nicotine probably does not share the inductive effect of tobacco on hepatic esterases/amidases necessary for the metabolism of aspirin and acetanilid.

*Acknowledgements*—We wish to thank Prof. K. P. Bhargava for advice and encouragement. Grateful acknowledgement is made to the Indian Council of Medical Research for awarding 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*

BASHEER ALI*
SWARAJ KAUR

REFERENCES

1. 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).
2. M. Inoue, M. Morikawa, M. Tsuboi, Y. Ito and M. Sugiura, *Jap. J. Pharmac.* **30**, 529 (1980).
3. R. Mentlein, S. Heiland and E. Heymann, *Archs Biochem. Biophys.* **200**, 547 (1980).
4. M. Järvinen, R. S. S. Santti and V. K. Hopsu-Havu, *Biochem. Pharmac.* **20**, 2971 (1971).
5. E. H. Silver and S. D. Murphy, *Toxic. appl. Pharmac.* **57**, 208 (1981).
6. K. Krisch, in *The Enzymes* (Ed. P. D. Boyer), Vol. 5, p. 43. Academic Press, New York (1971).
7. T. Satoh and K. Moroi, *Jap. J. Pharmac.* **27**, 233 (1977).
8. B. Ali, S. Kaur, A. Kumar and K. P. Bhargava, *Biochem. Pharmac.* **29**, 3087 (1980).
9. S. Kaur and B. Ali, *Biochem. Pharmac.* **31**, 1595 (1982).
10. P. Trinder, *Biochem. J.* **57**, 301 (1954).
11. R. M. Lee and B. H. Livett, *Biochem. Pharmac.* **16**, 1757 (1967).
12. J. A. Goldbarg, O. M. Friedman, E. P. Pineda, E. E. Smith, R. Chatterji, E. H. Stein and A. M. Rutenberg, *Archs Biochem. Biophys.* **91**, 61 (1950).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. K. Moroi and T. Kuga, *Biochem. Pharmac.* **29**, 1491 (1980).
15. K. Moroi and T. Satoh, *Biochem. Pharmac.* **24**, 1517 (1975).
16. E. Brandt, E. Heymann and R. Mentlein, *Biochem. Pharmac.* **29**, 1927 (1980).
17. W. S. Schwark and D. J. Ecobichon, *Can. J. Physiol. Pharmac.* **46**, 207 (1968).
18. U. Nousiainen and O. Hänninen, *Acta Pharmac. Toxic.* **49**, 77 (1981).

* To whom correspondence should be addressed.

*Biochemical Pharmacology*, Vol. 31, No. 22, pp. 3684–3687, 1982.
Printed in Great Britain.

0006-2952/82/223684-04 $03.00/0


## Clonidine effect on hepatic cGMP levels *in vivo* could be mediated by $\alpha_1$-adrenoceptors

(*Received* 12 *February* 1982; *accepted* 26 *April* 1982)

Clonidine has been used for some years as an $\alpha_2$-adrenergic agonist. Recent work from our laboratory has shown that, at low doses, clonidine administration produces a significant decrease in cerebellar cGMP levels due to its action on $\alpha_2$-adrenoceptors [1].

In parallel with the study of clonidine action in the