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COMPARATIVE STUDIES ON THE EFFECTS OF VARIOUS MICROSOMAL ENZYME INDUCERS ON THE *N*-DEMETHYLATION OF DIMETHYLNITROSAMINE

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Abstract—The effects of various compounds on the *N*-demethylation of dimethylnitrosamine (DMN) were investigated and grouped into three different categories according to their effects on both DMN-demethylase I and II (using 4 and 200 mM DMN concentrations respectively). 3-Methylcholanthrene (3-MC), β-naphthoflavone (β-NF), Aroclor 1254, benzo[*a*]pyrene (BP), and phenobarbital (PB) pretreatments decreased the activity of demethylase I, but they increased or had no effect on the activity of the second enzyme. Chlorpromazine pretreatment did not alter the activity of demethylase I whereas the activity of II was enhanced significantly. Feeding tryptophan and indole to rats, on the other hand, increased the activities of both demethylases as well as the cytochrome P-450 content.

It is generally recognized that dimethylnitrosamine (DMN), like most chemical carcinogens, must be activated by hepatic microsomal enzymes, DMN-demethylases, to exert its hepatotoxic and carcinogenic effects in a wide variety of mammalian species, including the rat [1]. Several reports indicate there are at least two demethylases, DMN-demethylase I and II, operating at low and high substrate concentrations, respectively, which accomplish the *N*-demethylation of DMN [2–5]. The demethylase activities can be altered by a variety of factors including drugs, foreign compounds, dietary components, and nutritional status [2–13]. In studies on the influence of some nutritional factors on the demethylation of DMN, it was found that tryptophan and indole affected the activity of DMN-demethylase I [5, 10, 11]. It was therefore decided to investigate further the influence of tryptophan and indole as well as other microsomal enzyme inducers [Aroclor 1254, phenobarbital (PB), β-naphthoflavone (β-NF), 3-methylcholanthrene (3-MC), benzo[*a*]pyrene (BP), and chlorpromazine hydrochloride (CPZ)] on both DMN-demethylases I and II. The results indicate that tryptophan and indole may represent a new class of microsomal enzyme inducers, the effects of which differ from those produced by the other compounds studied.

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MATERIALS AND METHODS

Animals. Young male Fischer rats (Charles River Breeding Laboratories, Inc., Wilmington, MA), weighing 60–100 g, were used. The animals were fed Wayne meal (Frederick Feed & Supply, Inc., Spring Valley, NY) and had free access to food and water unless otherwise stated.

Administration schedules. Aroclor 1254 (Monsanto, St. Louis, MO), was administered in corn oil, i.p., for 4 consecutive days, at a daily dose of 400 mg/kg body weight. β-NF (Aldrich Chemical Co., Milwaukee, WI) was administered i.p., in corn oil, for 2 consecutive days, at a daily dose of 80 mg/kg body weight. 3-MC (Eastman Organic Chemicals, Rochester, NY) and BP (Sigma Chemical Co., St. Louis, MO) were each administered as a single i.p. injection in corn oil at the dose level of 40 mg/kg body weight. PB (Mallinckrodt Chemical Works, St. Louis, MO) was administered in 0.9% NaCl, i.p., for 3 consecutive days at a daily dose of 75 mg/kg body weight. CPZ (a gift from Smith, Kline and French Laboratories, Philidelphia, PA) was administered in 0.9% NaCl as a single i.p. injection of 45 mg/kg body weight or as a daily dose of 20 mg/kg body weight for 6 days. The corresponding controls for each treatment received an equal volume of the vehicle. The animals were killed 24 hr after the last injection except in the repeated CPZ treatment where they were killed 2 hr after the last injection of the drug. L-Tryptophan (Fisher Chemical Co., Pittsburgh, PA) and indole (Calbiochem-Behring Co., La Jolla, CA) were mixed in Wayne meal to give a 1% concentration and were fed to rats for 8 days prior to killing them. The controls received Wayne meal alone.

Enzyme determinations. Animals were killed by decapitation. Livers were homogenized in 4 vol. (w/v) of 0.1 M potassium phosphate buffer, pH 7.4, and centrifuged for 20 min at 12,000 *g*. The supernatant fraction was centrifuged at 105,000 *g* for 60

min, producing the microsomal pellet which was suspended in 0.1 M potassium phosphate buffer, pH 7.4. Microsomal DMN-demethylases were determined as described earlier [2] using the method of Venkatesan *et al.* [14]. Substrate concentrations were 4 and 200 mM, which represented saturation levels for DMN-demethylases I and II respectively [3]. The amount of formaldehyde formed was determined by the method of Nash [15] with some modifications [16, 17]. The activity of the enzyme was expressed as nmoles of formaldehyde $\cdot hr^{-1} \cdot$ (mg microsomal protein)$^{-1}$. Cytochrome P-450 was determined by the method of Omura and Sato [18], using 91 $mM^{-1} \times cm^{-1}$ as molar extinction coefficient for the reduced cytochrome P-450–CO complex. Protein was determined by the method of Lowry *et al.* [19].

RESULTS

In these studies, the effects of various microsomal enzyme inducers on the DMN-demethylase system were classified into three different categories according to their effects on DMN-demethylases I and II.

Table 1 gives data on the compounds that depressed the activity of DMN-demethylase I but which either enhanced or had no effect on the activity of DMN-demethylase II. These compounds were Aroclor 1254, PB, β-NF, 3-MC, and BP. Pretreatment with 3-MC, β-NF, Aroclor 1254, BP, and PB reduced, in decreasing order of potency, the activity of DMN-demethylase I. DMN-demethylase II, on the other hand, responded differently under such conditions, for its activity was greatly enhanced by Aroclor 1254 and PB pretreatments. The enhancing ability, however, was much less in β-NF-pretreated rats. 3-MC and BP pretreatment had no effect on this enzyme. The liver: body weight ratio and microsomal protein content were greatly increased by Aroclor 1254 and, to a lesser extent, by PB pretreatment. Such changes were not observed in β-NF-, BP- and 3-MC-pretreated rats.

The effects of CPZ pretreatment differed from those of the compounds in Table 1. It caused no change in the activity of DMN demethylase I but a marked increase in the activity of DMN-demethylase II (Table 2). This effect was more pronounced when the animals were given a high dose of CPZ (45 mg/kg, as a single i.p. administration) than when a series of low doses (20 mg/kg, daily, for 6 days) was administered. However, neither single nor repeated CPZ pretreatment significantly altered the microsomal protein content and the liver: body weight ratio (Table 2).

Another type of effect was observed after feeding tryptophan or indole to rats at the 1% level for 8 days (Table 3). Contrary to the effects produced by the other substances, these compounds greatly enhanced DMN-demethylase I activity. Indole feeding resulted in a 262 per cent increase, whereas tryptophan feeding produced a 70 per cent increase. DMN-demethylase II responded in the same manner as DMN-demethylase I. The activity was increased by 111 and 33 per cent in indole- and tryptophan-fed rats respectively. The liver: body weight ratios were slightly increased in both cases, but the microsomal protein content was not affected. As can be seen from Table 4, the concentration of cytochrome P-450 increased during both tryptophan and indole feeding, but the increase was much higher in indole-fed rats.

DISCUSSION

The biotransformation of a carcinogen may yield either less harmful derivatives or active intermediates that are more toxic and carcinogenic. By far, the greatest number of such biotransformations are mediated through the mixed-function oxidases of the liver microsomes. Alterations in these enzyme activities, therefore, may affect both the toxicity and carcinogenicity of these compounds, as well as the rates at which various carcinogens are metabolized. The present studies on the effects of various microsomal enzyme inducers on the activities of both DMN-demethylases show that pretreatment of rats with Aroclor 1254, PB, and β-NF decreased DMN-demethylase I activity and markedly increased the activity of DMN-demethylase II. 3-MC and BP pretreatments decreased the activity of the first enzyme but did not alter significantly the activity of the second, in partial agreement with previous reports [3]. The microsomal enzyme inducers decrease the activity of DMN-demethylase I apparently by depressing *de novo* synthesis of enzyme [20]. The increases in liver: body weight ratio produced by Aroclor 1254 and PB may reflect the abilities of these compounds to increase the absolute weight of the liver, as well as the microsomal protein content. Thus, the increase in the liver weight may partially be a result of an actual increase in the *de novo* synthesis of hepatocyte proteins. Pretreatment of rats with β-NF, BP, and 3-MC did not alter either the liver: body weight ratio or the microsomal protein content significantly.

As reported previously, single or repeated CPZ pretreatment did not affect DMN-demethylase I activity but significantly increased the activity of DMN-demethylase II [2]. In our previous paper it was suggested that the induction of DMN-demethylase II might reflect a specific response of the CPZ-pretreated microsomal protein which, in turn, resulted in changes in the enzyme activity [2]. DMN and diethylnitrosamine at high concentrations, analogous to those for DMN-demethylase II, were reported to bring about conformational changes in proteins [21, 22].

The present work, as well as our previous reports [10, 11], showed that feeding either indole or tryptophan enhanced the activity of both DMN-demethylases. Arcos *et al.* [4], however, found that although tryptophan had such an effect, under their experimental conditions indole did not. The variation might be explained by the fact that they gave a single i.p. dose of indole 24 hr before killing the animals whereas in our study the rats were fed indole for 8 days. Nevertheless, the tryptophan or indole treatment, although increasing the liver: body weight ratio by 12–14 per cent, had no influence on hepatic microsomal protein content. The results thus suggest that tryptophan and indole may be examples of another class of microsomal enzyme inducers, since

Table 1. Effects of Aroclor 1254, phenobarbital, β-naphthoflavone, 3-methylcholanthrene and benzo[*a*]pyrene on hepatic DMN-demethylase activities in the rat

Treatment*	Liver:body weight ratio†		Microsomal protein† (mg/g liver)		DMN-demethylase I activity†,‡ [nmoles HCHO·hr^{-1} (mg microsomal protein)$^{-1}$]		DMN-demethylase II activity†,§ [nmoles HCHO·hr^{-1}·(mg microsomal protein)$^{-1}$]	
	Control	Exptl.	Control	Exptl.	Control	Exptl.	Control	Exptl.
Aroclor 1254 (400 mg/kg body wt, i.p., in corn oil, daily, for 4 days)	4.20 ± 0.11	7.10 ± 0.35 (69% increase) $P < 0.005$	11.80 ± 0.58	20.20 ± 0.69 (71% increase) $P < 0.005$	36.10 ± 2.35	15.11 ± 3.14 (58% decrease) $P < 0.005$	82.94 ± 6.25	208.52 ± 32.03 (151% increase) $P < 0.005$
Phenobarbital (75 mg/kg body wt, i.p., in saline, daily, for 3 days)	4.33 ± 0.12	5.89 ± 0.17 (36% increase) $P < 0.005$	13.98 ± 0.78	17.08 ± 0.69 (22% increase) $P < 0.01$	33.65 ± 3.31	26.00 ± 2.82 (23% decrease) $P < 0.005$	83.41 ± 5.80	205.62 ± 12.33 (147% increase) $P < 0.005$
β-Naphthoflavone (80 mg/kg body wt, i.p., in corn oil, daily, for 2 days)	4.18 ± 0.38	4.78 ± 0.48 No effect NS‖	11.80 ± 0.69	11.70 ± 0.51 No effect NS	39.82 ± 3.34	12.20 ± 1.11 (69% decrease) $P < 0.005$	88.86 ± 7.63	128.98 ± 15.40 (45% increase) $P < 0.025$
3-Methylcholanthrene (40 mg/kg body wt, i.p., in corn oil 24 hr prior to killing rats)	4.20 ± 0.15	4.50 ± 0.17 No effect NS	12.05 ± 0.72	13.02 ± 0.65 No effect NS	40.20 ± 3.35	11.38 ± 1.86 (72% decrease) $P < 0.005$	92.44 ± 5.82	103.12 ± 5.21 No effect NS
Benzo[*a*]pyrene (40 mg/kg body wt, i.p., in corn oil, 24 hr prior to killing rats)	3.94 ± 0.17	4.28 ± 0.19 No effect NS	13.00 ± 0.95	14.29 ± 0.67 No effect NS	48.00 ± 3.72	34.65 ± 3.31 (39% decrease) $P < 0.01$	116.11 ± 5.57	110.57 ± 5.45 No effect NS

* Control rats received an equivalent volume of the vehicle and were assayed together with the experimental rats.
† Values are the mean ± S.E. of six rats.
‡ A 4 mM concentration of DMN was used.
§ A 200 mM concentration of DMN was used.
‖ Value is not significant statistically.

Table 2. Effects of chlorpromazine pretreatment on hepatic DMN-demethylase activities in the rat

Treatment*	Liver:body weight ratio†‡		Microsomal protein† (mg/g liver)		DMN-demethylase I activity†§‖		DMN-demethylase II activity†§‖	
	Control	Exptl.	Control	Exptl.	Control	Exptl.	Control	Exptl.
Chlorpromazine (45 mg/kg body wt, i.p., in saline, 24 hr prior to killing rats)	4.08 ± 0.10	4.28 ± 0.12	13.13 ± 0.82	14.22 ± 0.93	40.21 ± 3.10	38.52 ± 4.21	91.82 ± 8.20	158.42 ± 13.21
	No effect NS**		No effect NS		No effect NS		(73% increase) $P < 0.005$	
Chlorpromazine (20 mg/kg body wt, i.p., in saline, daily, for 6 days)	4.18 ± 0.11	4.37 ± 0.14	13.48 ± 0.94	14.32 ± 0.88	44.21 ± 4.81	40.24 ± 6.22	74.88 ± 9.10	114.22 ± 10.21
	No effect NS		No effect NS		No effect NS		(53% increase) $P < 0.01$	

* Controls received an equivalent volume of vehicle and were assayed together with the experimental rats.
† Values are the means ± S.E. of seven rats.
‡ Ratios are g liver:g body wt.
§ Activity is expressed as nmoles $HCHO \cdot hr^{-1} \cdot (mg\ microsomal\ protein)^{-1}$.
‖ A 4 mM concentration of DMN was used.
¶ A 200 mM concentration of DMN was used.
**Value is not significant statistically.

Table 3. Effects of tryptophan and indole feeding on hepatic DMN-demethylase activities in the rat

Treatment*	Liver:body weight ratio†‡		Microsomal protein† (mg/g liver)		DMN-demethylase I activity†§‖		DMN-demethylase II activity†§¶	
	Control	Exptl.	Control	Exptl.	Control	Exptl.	Control	Exptl.
L-Tryptophan (1% in the diet for 8 days, prior to killing rats)	4.20 ± 0.09	4.70 ± 0.10	12.23 ± 0.84	13.18 ± 0.58	42.81 ± 3.21	72.85 ± 6.10	96.98 ± 9.21	129.22 ± 12.78
	(12% increase) $P < 0.005$		No effect NS**		(70% increase) $P < 0.005$		(33% increase) $P < 0.05$	
Indole (1% in the diet for 8 days, prior to killing rats)	4.23 ± 0.13	4.82 ± 0.15	13.08 ± 0.58	14.42 ± 0.66	36.71 ± 4.28	132.80 ± 10.36	84.61 ± 9.21	178.32 ± 13.68
	(14% increase) $P < 0.01$		No effect NS		(262% increase) $P < 0.005$		(111% increase) $P < 0.005$	

* Controls were fed Wayne meal.
† Values are the means ± S.E. of eight rats.
‡ Ratios are g liver:g body wt.
§ Activity is expressed as nmoles $HCHO \cdot hr^{-1} \cdot (mg\ microsomal\ protein)^{-1}$.
‖ A 4 mM concentration of DMN was used.
¶ A 200 mM concentration of DMN was used.
** Value is not significant statistically.

Table 4. Effects of tryptophan and indole feeding on the cytochrome P-450 content of hepatic microsomes of the rat

Treatment*	No. of rats	Cytochrome P-450† (nmoles/mg microsomal protein)
Control	8	1.05 ± 0.09
Tryptophan	8	1.34 ± 0.11 (28% increase) $P < 0.05$
Indole	8	1.51 ± 0.13 (44% increase) $P < 0.01$

* Rats were fed tryptophan and indole, at a 1% concentration level in the diet, for 8 days before they were killed.

† Values are means ± S.E.

their effects were opposite to those of the other enzyme inducers.

Although there is strong evidence to indicate that the demethylation of DMN is cytochrome P-450 dependent [23], other mechanisms have been proposed [24], including the participation of a monoamine oxidase [25]. However, the present finding that tryptophan and indole feeding greatly increased the amount of cytochrome P-450 as well as the activity of DMN-demethylases suggests that demethylation may involve cytochrome P-450.

The results of these studies demonstrated the abilities of various substances to alter the DMN-metabolizing enzyme activities. Some of these chemicals are used as drugs, e.g. CPZ and PB; others are environmental compounds, e.g. BP and Aroclor 1254. Even dietary components such as tryptophan and indole can enhance the activities of these enzymes significantly. All these substances are likely to be encountered by man. Since DMN requires metabolic activation for its hepatotoxic and carcinogenic actions, alterations in the activities of the metabolizing enzymes may change susceptibility to the carcinogenicity of DMN.

REFERENCES

1. P. N. Magee and J. M. Barnes, *Adv. Cancer Res.* **10**, 163 (1967).
2. M. H. Mostafa and E. K. Weisburger, *J. natn. Cancer Inst.* **64**, 925 (1980).
3. J. C. Arcos, D. L. Davies, C. E. L. Brown and M. F. Argus, *Z. Krebsforsch.* **89**, 181 (1977).
4. J. C. Arcos, S. C. Myers, B. J. Neuburger and M. F. Argus, *Cancer Lett.* **9**, 161 (1980).
5. L. M. Anderson and M. Angel, *Biochem. Pharmac.* **29**, 1375 (1980).
6. J. D. Judah, A. E. M. McLean and E. K. McLean, *Am. J. Med.* **49**, 609 (1970).
7. A. E. M. McLean and H. G. Verschuuren, *Br. J. exp. Path.* **50**, 22 (1969).
8. D. Schmähl, F. W. Krüger, S. Ivankovic and P. Preissler, *Arzneimittel-Forsch.* **21**, 1560 (1971).
9. B. Stripp, M. E. Hamrick and J. R. Gillette, *Biochem. Pharmac.* **21**, 745 (1972).
10. R. P. Evarts and M. H. Mostafa, *Fd. Cosmet. Toxic.* **16**, 585 (1978).
11. R. P. Evarts and M. H. Mostafa, *Proc. Am. Ass. Cancer Res.* **20**, 188 (1979).
12. M. Ruchirawat, W. Mahathanatrakul, Y. Sinrashatanant and D. Kittikook, *Biochem. Pharmac.* **27**, 1783 (1978).
13. J. J. Hutton, C. Hackney and J. Meier, *Mutation Res.* **64**, 363 (1979).
14. N. Venkatesan, J. C. Arcos and M. F. Argus, *Life Sci.* **7**, 1111 (1968).
15. T. Nash, *Biochem. J.* **55**, 416 (1953).
16. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
17. A. E. M. McLean and P. A. Day, *Biochem. Pharmac.* **23**, 1173 (1974).
18. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. M. F. Argus, J. C. Arcos, K. M. Pastor, B. C. Wu and N. Venkatesan, *Chem. Biol. Interact.* **13**, 127 (1976).
21. M. F. Argus, J. C. Arcos, J. H. Mathison, A. Alam and J. A. Bemis, *Arzneimittel-Forsch.* **16**, 740 (1966).
22. J. A. Bemis, M. F. Argus and J. C. Arcos, *Biochim. biophys. Acta* **126**, 274 (1966).
23. P. Czygan, H. Greim, A. J. Garro, F. Hutterer, F. Schaffner, H. Popper, O. Rosenthal and D. Y. Cooper, *Cancer Res.* **33**, 2983 (1973).
24. B. G. Lake, C. E. Heading, J. C. Phillips, S. D. Gangolli and A. G. Lloyd, *Biochem. Soc. Trans.* **2**, 610 (1974).
25. I. R. Rowland, B. G. Lake, J. C. Phillips and S. D. Gangolli, *Mutation Res.* **72**, 63 (1980).