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NICOTINAMIDE-INDUCED HEPATIC MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM IN RATS

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Summary

A single intraperitoneal injection of nicotinamide (100 mg/kg body wt.), an endogenous non-toxic metabolite, to male rats was shown to significantly induce all the components of the hepatic microsomal mixed function oxidase system such as NADPH-cytochrome *c* reductase, cytochrome *P*-450 and cytochrome b_5 as well as activities of drug-metabolizing enzymes such as arylhydrocarbon hydroxylase, aminopyrine demethylase and UDPglucuronosyltransferase. Tryptophan (1000 mg/kg body wt.) and nicotinic acid (200 mg/kg body wt.) as well as methionine (200 mg/kg body wt.) were also shown to be effective inducers in terms of hepatic NADPH-cytochrome *c* reductase activity. The nicotinamide-induced mixed function oxidase system was shown to be associated with an increased incorporation of ^{14}C-labelled leucine into hepatic microsomal proteins which was inhibited by puromycin. Nicotinamide was shown to induce a distinct pattern of drug-metabolizing system in comparison with that of phenobarbital.

Introduction

A variety of structurally unrelated foreign compounds including drugs, carcinogens and environmental chemicals are known to induce the hepatic microsomal drug-metabolizing enzymes which have been extensively reviewed in recent years [1—4]. Several naturally occurring nutrients such as terpenes [5], coumarins [6], flavones [7,8] and caffeine [9] have also been shown to

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enhance the activity of the mixed function oxidases. Among the endogenous substrates, certain steroid hormones as well as sterols including cholesterol [1, 10,11] have been known to induce these enzymes so far. During our studies on the possible influence of pyridine nucleotide levels on the activity of NADPH-cytochrome *c* reductase during foetal development in rats, it was revealed that an injection of nicotinamide, a precursor of pyridine nucleotides, brought about a significant increase in the activity of this enzyme in the maternal liver. Following this observation it was demonstrated that a single intraperitoneal injection of nicotinamide to adult rats in a dose lower than that required for the synthesis of pyridine nucleotides, also induced significant increases in the hepatic NADPH-cytochrome *c* reductase activity as well as in other components of the mixed function oxidase system, such as cytochrome *P*-450 and cytochrome b_5. The present paper relates to these observations. The induction pattern by nicotinamide, a relatively non-toxic normal metabolite, was compared with that obtained by multiple administration of phenobarbital, a toxic drug. Nicotinamide administration to rats was also shown to induce activities of hepatic arylhydrocarbon hydroxylase, aminopyrine demethylase as well as UDPglucuronosyltransferase. An induction of NADPH-cytochrome *c* reductase activity was also demonstrated following administration of tryptophan, nicotinic acid as well as methionine to rats in independent experiments.

Materials and Methods

Chemicals. Nicotinamide adenine dinucleotide phosphate (reduced form; NADPH), cytochrome *c*, uridine 5′-diphosphoglucuronic acid, 3,4-benzo(*a*)-pyrene, nicotinamide, puromycin, DL-tryptophan, DL-methionine and the detergent Triton X-100 were purchased from Sigma Chemical Company, St. Louis; 4-dimethylaminoantipyrine (aminopyrine) was obtained from Aldrich Chemical Company, Milwaukee; phenobarbital was obtained from Indian Drugs and Pharmaceuticals Ltd., Hyderabad; nicotinic acid was obtained from S.B. Penick Company, New York. DL-[1-^{14}C]Leucine (48.6 Ci/mol) was obtained from Isotope Division, Bhabha Atomic Research Centre, Bombay, and all other chemicals used were obtained from BDH Chemicals Division, Glaxo Laboratories (India) Ltd., Bombay.

Methods. Adult Wistar rats, males and females (weighing between 150 and 200 g fed ad libitum on laboratory diet were used in these experiments. Nicotinamide was administered intraperitoneally in various single doses ranging from 50 to 500 mg/kg body wt. to male and female rats which were killed at various intervals of time. Tryptophan (200, 500, 1000 mg/kg body wt.) and nicotinic acid (100, 150, 175, 200 mg/kg body wt.) were administered intraperitoneally to male rats 24 h before killing. Sodium phenobarbital was administered intraperitoneally to male rats in a daily dose of 100 mg/kg body wt. for 5 days and the rats were killed 24 h after the last injection. DL-Methionine was administered intraperitoneally in a single dose of 200 mg/kg body wt. 24 h before killing.

For assays of cytochrome *P*-450 and cytochrome b_5, livers were perfused with ice-cold physiological saline prior to excision and homogenates were prepared in 0.154 M KCl. For the enzyme assays, liver homogenates were

prepared in 4 vols. of 0.25 M sucrose containing 0.01 M $MgCl_2$, except that of UDPglucuronosyltransferase in which case the homogenate was prepared in 0.154 M KCl. Hepatic microsomal preparations were obtained according to the method of Berezney et al. [12].

The concentrations of cytochrome *P*-450 and cytochrome b_5 were determined as described by Isselbacher et al. [16] with *p*-nitrophenol as the substrate. activity was measured according to Williams and Kamin [14]. The reduction of cytochrome *c* was calculated on the basis of molar absorbance indices given by Margoliash and Frohwirt [15]. UDPglucuronosyltransferase activity was determined as described by Isselbacher et al. [16] with *p*-nitrophenol as the substrate. Arylhydrocarbon hydroxylase was assayed according to the procedure of Nebert and Gelboin [17] using 3,4-benzo(*a*)pyrene as its substrate, and activity of aminopyrine demethylase was measured by the method of Cochin and Axelrod [18]. Estimation of microsomal protein was carried out essentially according to the procedure of Lowry et al. [19] using crystalline bovine serum albumin as the standard.

Effect of nicotinamide and puromycin on the incorporation of ^{14}C-labelled leucine into hepatic microsomal proteins. Incorporation of ^{14}C-labelled leucine into total hepatic microsomal proteins was studied 24 h after nicotinamide administration to male rats (100 mg/kg body wt. intraperitoneally). Puromycin (7.5 mg/100 g body wt.) was administered intraperitoneally to rats immediately after nicotinamide in independent experiments. DL-[1-^{14}C]Leucine (5 μCi/100 g body wt.) was administered intraperitoneally to rats 30 min before killing. Hepatic microsomal suspension (0.2 ml) was spotted on Whatman filter paper (3 mm thick) strips (7 cm × 1.8 cm) which were further processed by the method of Mans and Novelli [20] as modified by Roodyn et al. [21].

Results and Discussion

Fig. 1 gives the dose-response curve at various intervals for nicotinamide-induced hepatic microsomal NADPH-cytochrome *c* reductase activity in male and female rats. Whereas the optimal induction is obtained at 24 h following nicotinamide administration to both male and female rats, it can be seen that the dose required for females (250 mg/kg body wt.) is 2.5 times the dose needed for males (100 mg/kg body wt.) to obtain a significant enzyme induction. Nicotinamide administered to female rats in a lower dose (100 mg/kg body wt.) failed to induce the enzyme activity in liver. The reason for the difference in the induction doses of nicotinamide between male and female rats is not yet clear although sex differences in the metabolism of drugs are well known [1,22,23].

As seen in Table I, both, tryptophan and nicotinic acid are also effective in bringing about induction of hepatic microsomal NADPH-cytochrome *c* reductase activity in male rats. Whereas the inductive dose of tryptophan is quite high (1000 mg/kg body wt.) as could be expected, nicotinic acid gave a comparable response with twice the dose of nicotinamide. These results could be expected since nicotinamide as well as nicotinic acid are products resulting from the metabolism of tryptophan. It would appear that the induction of

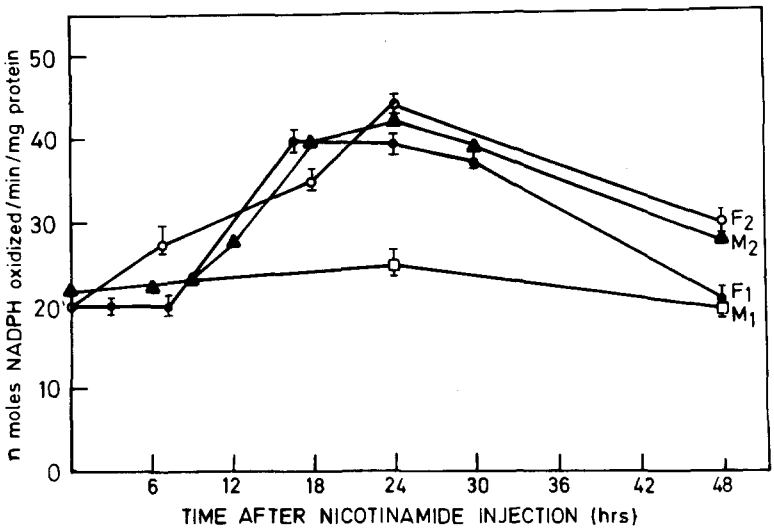

Fig. 1. Hepatic NADPH-cytochrome *c* reductase activity in male and female rats treated with different doses of nicotinamide. Different doses of nicotinamide were injected intraperitoneally to male (□———□, 50 mg/kg body wt.; ▲———▲, 100 mg/kg body wt.) and female rats (●———●, 250 mg/kg body wt.; ○———○, 500 mg/kg body wt.) and NADPH-cytochrome *c* reductase activity was determined in rat liver microsomal fractions at various intervals up to 48 h.

NADPH-cytochrome *c* reductase activity may not be related to pyridine nucleotide levels since 24 h following nicotinamide administration, the hepatic pyridine nucleotide levels have been shown to return to the control values [24]. Moreover, no significant increase in the pyridine nucleotide levels has been demonstrated [25] by injection of nicotinamide at a dose level of 100 mg/kg body wt.

Levels of other components of mixed function oxidase system such as cyto-

TABLE I

HEPATIC MICROSOMAL NADPH-CYTOCHROME *c* REDUCTASE ACTIVITY FOLLOWING ADMINISTRATION OF NICOTINIC ACID AND TRYPTOPHAN TO MALE RATS

Nicotinic acid in doses ranging from 50 to 200 mg/kg body wt. and DL-tryptophan in doses ranging from 100 to 1000 mg/kg body wt. were intraperitoneally administered to male rats and hepatic microsomal NADPH-cytochrome *c* reductase activity was determined 24 h after the treatment. Values represent mean ± S.E. Figures in parentheses indicate the number of rats used for each observation.

Treatment: nicotinic acid (mg/kg body wt.)	NADPH-cytochrome *c* reductase activity (nmol NADPH oxidized/min per mg microsomal protein)	Treatment: DL-tryptophan (mg/kg body wt.)	NADPH-cytochrome *c* activity (nmol NADPH oxidized/min per mg microsomal protein)
0 (control)	21.5 ± 4 (15)	0 (control)	21.5 ± 4 (15)
50	27 ± 3 (4)	100	23 ± 2 (6)
100	33 ± 4 (4)	200	23 ± 1.5 (4)
150	36.5 ± 1.5 (4)	500	23 ± 1.5 (4)
175	36.5 ± 1 (4)	1000	46.5 ± 7 (4)
200	39.5 ± 2 (4)		

chrome *P*-450 and cytochrome b_5 are increased in rat liver following administration of nicotinamide (Table II). The increased levels of these components following multiple injections of phenobarbital are also given for comparison. It is evident that the increased activity of NADPH-cytochrome *c* reductase as well as the increased levels of cytochrome *P*-450 following nicotinamide administration are well comparable with those obtained after multiple injections of phenobarbital. With the exception of cytochrome b_5, a higher induction (4.5-fold) of these hepatic microsomal components by phenobarbital using Sprague-Dawley rats was reported by Orrenius et al. [26] as well as by others. However, in the present experiments using Wistar rats, the maximum induction obtained even with multiple doses of phenobarbital was only about 2—2.5 times the control values, the increase being similar to that obtained by a single dose of nicotinamide. The induction pattern with nicotinamide is better than that obtained with phenobarbital with respect to cytochrome b_5. Whereas no significant induction of cytochrome b_5 could be obtained after multiple injections of phenobarbital, a single dose of nicotinamide was sufficient to bring a 70% increase of this cytochrome. A slight increase in the levels of cytochrome b_5 following phenobarbital administration has been reported by other workers [27,28], although no such increase in cytochrome b_5 levels following phenobarbital administration could be obtained by Orrenius et al. [26] as well as in the present experiments. Recent reports have suggested that cytochrome b_5 is possibly involved in the cytochrome *P*-450-dependent microsomal hydroxylation [29—31]. In this context, the observed increase of hepatic cytochrome b_5 following nicotinamide administration to rats is interesting.

An increase in the incorporation of DL-[1-^{14}C]leucine into hepatic microsomal proteins was observed at 24 h following nicotinamide administration to rats (Table III). The sensitivity of the increased ^{14}C-labelled leucine incorporation to puromycin suggests increased protein synthesis following nicotinamide administration. Similar effect of puromycin, on the nicotinamide-induced hepatic NADPH-cytochrome *c* reductase activity was also obtained. It was shown that a 20-fold increase in activity of the hepatic enzyme at 24 h following nicotinamide (100 mg/kg body wt.) administration to rats was completely

TABLE II

HEPATIC MICROSOMAL MIXED FUNCTION OXIDASE COMPONENTS FOLLOWING ADMINISTRATION OF NICOTINAMIDE AND PHENOBARBITAL TO MALE RATS

Nicotinamide (100 mg/kg body wt. single dose) and phenobarbital (100 mg/kg body wt. daily for 5 days) were intraperitoneally administered to male rats in independent experiments and hepatic microsomal NADPH-cytochrome *c* reductase activity as well as levels of cytochrome *P*-450 and cytochrome b_5 were determined 24 h after the treatment. Values represent mean ± S.E. Figures in parentheses indicate the number of rats used for each observation.

Treatment	NADPH-cytochrome *c* reductase activity (nmol NADPH oxidized/min per mg microsomal protein)	Cytochrome *P*-450 (nmol/mg microsomal protein)	Cytochrome b_5 (nmol/mg microsomal protein)
Control	21.5 ± 4 (15)	0.81 ± 0.07 (10)	0.61 ± 0.05 (10)
Nicotinamide	42 ± 4 (12)	1.38 ± 0.05 (8)	1.04 ± 0.05 (8)
Phenobarbital	43 ± 6 (8)	1.26 ± 0.13 (5)	0.67 ± 0.05 (5)

TABLE III

EFFECT OF PUROMYCIN ON THE INCORPORATION OF ^{14}C-LABELLED LEUCINE IN HEPATIC MICROSOMAL PROTEINS FOLLOWING ADMINISTRATION OF NICOTINAMIDE TO RATS

The incorporation of DL-[1-^{14}C]leucine in hepatic microsomal proteins was studied 24 h after nicotinamide (100 mg/kg body wt.) administration to male rats. Puromycin (7.5 mg/100 g body wt.) was administered to rats immediately following nicotinamide in an independent experiment. ^{14}C-Labelled leucine (5 μCi/100 g body wt.) was administered to rats 30 min before killing. The values are the averages of three independent experiments; figures in parentheses indicate the range.

Treatment	cpm/mg microsomal protein
None	274 (240—350)
Nicotinamide	524 (456—620)
Nicotinamide + puromycin	245 (210—300)

abolished by simultaneous administration of puromycin (7.5 mg/100 g body wt.). These observations are similar to those reported for phenobarbital by Orrenius et al. [26] who showed that the increased enzyme activity following phenobarbital administration to rats was inhibited by puromycin. It was further shown [32] that phenobarbital increased the incorporation, both in vitro and in vivo, of labelled amino acids into microsomal proteins. Cohen and Ruddon [33] as well as Smith et al. [34] suggested that phenobarbital administration resulted in increased post-transcriptional stabilization of the rat hepatic nuclear ribosomal-RNA precursors rather than enhancing its synthesis. Further, phenobarbital was shown to decrease the activities of ribonuclease in all the hepatic subcellular fractions [35] and thus, to increase the stability of rat liver polyribosomes [36]. Such studies have to be carried out with nicotinamide for further evaluation of its mechanism of action.

Table IV gives the increased activity of hepatic microsomal drug-metabolizing enzymes such as arylhydrocarbon hydroxylase and aminopyrine

TABLE IV

EFFECT OF NICOTINAMIDE INJECTION ON LIVER MICROSOMAL DRUG-METABOLIZING ENZYMES IN RATS

Hepatic microsomal activities of UDPglucuronosyltransferase, arylhydrocarbon hydroxylase and aminopyrine demethylase were determined 24 h after an intraperitoneal injection of nicotinamide to adult male rats. In case of UDPglucuronosyltransferase activity the microsomal preparations were further solubilized with detergent Triton X-100 (0.1%). Figures in brackets indicate enzyme activity after the detergent treatment. Values represent mean ± S.E. Figures in parentheses indicate the number of rats used for each observation. Units of arylhydrocarbon hydroxylase activity are changes in fluorescence reading at 395 and 522 nm.

Treatment	UDPglucuronosyltransferase activity (μmol of *p*-nitrophenol conjugated/min per mg microsomal protein)	Arylhydrocarbon hydroxylase activity (units/mg microsomal protein)	Aminopyrine demethylase activity (nmol of formaldehyde formed/min per mg microsomal protein)
None	4.2 ± 0.44 (10) [5.98 ± 0.29]	20.7 ± 0.57 (12)	9.3 ± 1.1 (15)
Nicotinamide	7.4 ± 0.12 (10) [9.78 ± 0.33]	45.5 ± 1.6 (10)	17.5 ± 0.75 (15)

demethylase as well as UDPglucuronosyltransferase, the enzyme which catalyses conjugation of many xenobiotics and their metabolites with UDP-glucuronic acid. It can be seen that both, arylhydrocarbon hydroxylase as well as aminopyrine demethylase activities are about 2-fold at 24 h following nicotinamide administration. Most striking is the effect of nicotinamide on the activity of UDPglucuronosyltransferase in comparison with that of phenobarbital. Whereas phenobarbital is known to increase the latency of this enzyme [37], the enhanced activity being observed only after detergent treatment [38], nicotinamide is shown to bring about an increase of 80% in the activity of this enzyme irrespective of the detergent treatment, thereby demonstrating that the increase in enzyme activity is directly manifested. There is a 50% increase in the enzyme activity following treatment of hepatic microsomal preparation with Triton X-100 in both, the control as well as the nicotinamide-treated rats. The polycyclic hydrocarbons 3-methylcholanthrene and 3,4-benzo(*a*)pyrene are also known to induce UDPglucuronosyltransferase activity which is expressed without the detergent treatment [39]. The induction of this enzyme by nicotinamide resembles that by the polycyclic hydrocarbons, whereas induction of NADPH-cytochrome *c* reductase activity as well as of cytochrome *P*-450 by nicotinamide is similar to the induction of these components by phenobarbital. Again, as in the case of polycyclic hydrocarbons [3], there was no significant increase in the hydroxylation of pentobarbital following nicotinamide administration to rats (data not included). In comparison with the induction pattern reported for phenobarbital and hydrocarbons, it appears that nicotinamide exhibits a different pattern of induction.

The induction of mixed function oxidases by nicotinamide is intriguing since metabolism of this substrate to N_1-methylnicotinamide and further oxidation to excretable pyridone in many mammalian species is not known to involve microsomal hydroxylation. Since the methyl groups for the methylation of nicotinamide originate from methionine through *S*-adenosylmethionine the effect of methionine administration (200 mg/kg body wt. intraperitoneally) to rats on hepatic NADPH-cytochrome *c* reductase activity was investigated. As seen in Table V, methionine is able to bring about a significant induction of hepatic NADPH-cytochrome *c* reductase activity which is comparable with the

TABLE V

HEPATIC MICROSOMAL NADPH-CYTOCHROME *c* REDUCTASE ACTIVITY FOLLOWING ADMINISTRATION OF NICOTINAMIDE AND METHIONINE TO MALE RATS

Nicotinamide (100 mg/kg body wt.) and DL-methionine (200 mg/kg body wt.) were intraperitoneally administered, singly and in combination, to male rats in independent experiments. Hepatic microsomal NADPH-cytochrome *c* reductase activity was determined 24 h after the treatment. Values represent mean ± S.E. Figures in parentheses indicate the number of rats used for each observation.

Treatment	NADPH-cytochrome *c* reductase activity (nmol NADPH oxidized/min per mg microsomal protein)
Control	21.5 ± 4 (15)
Nicotinamide	42 ± 4 (12)
Methionine	40.6 ± 4 (4)
Nicotinamide + methionine	54.3 ± 3 (4)

induced enzyme activity by nicotinamide. When nicotinamide and methionine were administered together, there was a further rise in the induction of the enzyme activity.

Methionine is a methyl donor through *S*-adenosylmethionine in many biosynthetic pathways. Of particular interest, in this respect, is the methylation of γ-aminobutyric acid to form γ-butyrobetaine followed by its hydroxylation through the mixed function oxidase system to form carnitine which participates in the mitochondrial fatty acid transport. It may be that formation of γ-butyrobetaine by methionine administration induces the mixed function oxidase activity. Whether N_1-methylnicotinamide is also implicated in NADPH-dependent microsomal hydroxylation is not yet clear.

Further work would be necessary to elucidate the mechanism of induction of the hepatic microsomal mixed function oxidases by nicotinamide. However, the increased activities of drug-metabolizing enzymes by endogenous non-toxic metabolites may have therapeutic implications in reducing the toxicity of drugs, particularly, in conditions in which the drug-metabolizing capacity is at a subnormal level. In this respect, nicotinamide was shown to correct the impaired hepatic drug metabolism in tumour-bearing animals (Narurkar, M.V. and coworkers, unpublished results). Further, nicotinamide was also shown to bring about a significant induction of the hepatic supernatant glutathione-*S*-aryltransferase within 6 h after its administration to rats (Narurkar, M.V. and coworkers, unpublished results).

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