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INHIBITORY EFFECTS OF NIACIN AND ITS ANALOGUES ON INDUCTION OF ORNITHINE DECARBOXYLASE ACTIVITY BY DIETHYLNITROSAMINE IN RAT LIVER

MITSUAKI SAKAMOTO, SUSUMU YANAGI* and TOMOYA KAMIYA
Department of Biochemistry, Nara Medical College, Nara 634, Japan

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Abstract—Pharmacological doses of niacin and its analogues were given intraperitoneally to rats with and without coadministration of a hepatocarcinogenic dose of diethylnitrosamine (DEN), and their effects on the induction of ornithine decarboxylase (ODC, EC 4.1.1.17) activity in the rat liver were studied. The induction of ODC activity by DEN was inhibited by 74.3, 85.5, 94.6, 97.6, 72.6 and 55.2% by nicotinamide, nicotinic acid, 3-hydroxymethylpyridine, β-picoline, pyridine-3-aldehyde and ethylnicotinate respectively. When given alone, these analogues did not induce ODC activity. All these compounds are known to have a niacin effect. DEN-induced ODC activity was also inhibited by 84.0, 93.3, 52.8 and 75.9% by 6-aminonicotinamide, picolinic acid, pyridine-3-sulfonic acid and thionicotinamide, respectively, but, peculiarly, they induced ODC activity by their administration alone. These niacin analogues are known to have anti-niacin effects. Tryptophan, *N'*-methylnicotinamide and isonicotinic acid hydrazide did not affect the DEN-induced ODC activity but could induce ODC by themselves. Tryptophan belongs to the former group and isonicotinic acid hydrazide to the latter group. The reason for these discrepancies is discussed.

Aside from its role as a vitamin, niacin and its analogues are known to have various pharmacological effects when doses in excess of nutritional requirements are given [1]. In 1980, Wagner *et al.* [2] reported that an intraperitoneal administration of nicotinamide to rats inhibited induction by secobarbital of liver ODC† activity. This report attracted our attention and raised the following questions: (1) Does nicotinamide inhibit ODC activity induced by means other than barbiturates? (2) Can its effect on ODC be explained by its inhibitory effects on poly(ADP-ribose) synthesis? (3) Do the modes of action of niacin analogues correlate with either niacin or anti-niacin effects?

The present report deals with these questions and gives some answers to them as well as supporting evidence relating the induction of liver ODC to NAD metabolism.

MATERIALS AND METHODS

Chemicals. Nicotinamide, nicotinic acid, tryptophan, quinolinic acid, *o*- and *p*-aminobenzamide, thionicotinamide, pyridine-3-sulfonic acid, β-picolin and benzamide were obtained from Nakarai Chemicals Ltd., Kyoto. Picolinic acid, pyridine-3-aldehyde, 3-acetylpyridine, 3-hydroxymethylpyridine, isonicotinic acid hydrazide and DEN were obtained from Wako Pure Chemical Industries, Ltd., Osaka. Ethylnicotinate and *m*-aminobenzamide were obtained from the Tokyo Kasei Kogyo Co., Ltd., Tokyo. *N'*-Methylnicotinamide and 6-aminonicotinamide were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. D,L-[1-^{14}C]Ornithine (50 mCi/mmol) was obtained from New England Nuclear, Boston, MA, U.S.A.

Animals. Male Wistar rats were obtained from the Kiwa Laboratory Animals Co., Wakayama, at 5 weeks of age. They were maintained on a commercial diet (MF, Oriental Yeast Co., Tokyo) in an air-conditioned room at 22 ± 1° under natural light conditions. Food and water were given *ad lib.* The composition of the diet has been described previously [3]. After 1 week of accommodation, the rats were fasted overnight. The enzyme activity was induced by a single injection of DEN (200 mg/kg body wt). The ability of a particular chemical to suppress enzyme induction was tested by simultaneous intraperitoneal injections of the chemical and DEN. The test compounds were dissolved in distilled water or dimethyl sulfoxide. The doses [mmol·(3 ml vehicle)$^{-1}$·(kg body wt)$^{-1}$] of the compounds administered were as follows: 7.5, nicotinamide, nicotinic acid, ethylnicotinate, *p*-aminobenzamide and pyridine-3-aldehyde; 3.75, *o*-aminobenzamide, 3-hydroxymethylpyridine and β-picolin; 3.00, pyridine-3-sulfonic acid and thionicotinamide; 2.00, 6-aminonicotinamide, *N'*-methylnicotinamide and isonicotinic acid hydrazide; 1.80, tryptophan, quinolinic acid and picolinic acid; 0.90, 3-acetylpyridine; and 0.75, benzamide and *m*-aminobenzamide respectively. These amounts were maximal doses in order not to induce an acute toxic effect in the rats.

Enzyme assay. Animals were killed by decapitation 4 hr after the injection of the chemicals. The liver was removed quickly and homogenized with a Potter–Elvehjem homogenizer in 2 vol. (v/w) of 0.1 M sodium phosphate buffer (pH 7.5) containing 0.8 mM pyridoxal phosphate and 2 mM ethylenediamine tetraacetic acid [4]. The homogenates obtained were centrifuged at 12,000 *g* for 1 hr, and

* Address all correspondence to: Dr. Susumu Yanagi, Department of Biochemistry, Nara Medical College, Shijo-cho Kashihara, Nara 634, Japan.

† Abbreviations: ODC ornithine decarbopylase; DEN, diethylnitrosamine; and NAD, nicotinamide adenine dinucleotide.

Table 1. Inhibitory effect of nicotinamide on ODC and tyrosine aminotransferase induction by DEN

Experimental group	ODC Activity [nmol·min^{-1}·(g liver)$^{-1}$]	Inhibition (%)	Tyrosine aminotransferase activity [μmol·min^{-1}·(g liver)$^{-1}$]
Control	0.50 ± 0.07 (5)	—	1.98 ± 0.10 (5)
Nicotinamide	0.39 ± 0.08* (5)	—	19.06 ± 0.21*,† (5)
DEN	12.06 ± 1.98† (5)	0	11.88 ± 0.85† (5)
DEN + nicotinamide	2.76 ± 0.69*,‡ (5)	80.4	13.53 ± 0.88† (5)

Male rats were fasted overnight. They received an intraperitoneal injection of DEN (200 mg/kg body wt) and/or nicotinamide (7.5 mmol/kg body wt) 4 hr before being killed. Values are mean ± SEM with the number of animals given in parentheses.

* Significantly different from DEN group, $P < 0.005$.

†,‡ Significantly different from control group: † $P < 0.001$, and ‡ $P < 0.05$.

the resultant supernatant fractions were used for the enzyme assays. Tyrosine aminotransferase (EC 2.6.1.5) activity was assayed as described previously [4]. ODC activity was determined by measuring the liberation of $^{14}CO_2$ and is expressed as nmol per min per g wet weight of the liver at 37°. The reaction mixture was the same as described previously [4]. The reaction was carried out in a conical flask with a center well for 30 min at 37° in a shaking water bath. The evolved CO_2 was trapped by a piece of filter paper soaked in 0.05 ml of 10% (w/v) KOH in the center well. The reaction was stopped by an injection of 0.4 ml of 2.5 M citric acid. The reaction flasks were further incubated at 37° for an additional hour. The radioactivity of the filter paper was determined by a liquid scintillation counter. The inhibitory effect of chemicals on hepatic ODC activity induction by DEN was calculated by the following equation:

$$\frac{(\text{DEN}) - (\text{DEN} + \text{a chemical})}{(\text{DEN}) - (\text{control})} \times 100$$

where (control) = hepatic ODC activity in untreated rats, (DEN) = hepatic ODC activity in 4 hr after DEN injection, and (DEN + a chemical) = hepatic ODC activity 4 hr after the simultaneous injection of DEN and a chemical.

Induction of ODC activity by a chemical was compared with that by DEN and calculated as follows;

$$\frac{(\text{a chemical}) - (\text{control})}{(\text{DEN}) - (\text{control})} \times 100$$

where (a chemical) = ODC activity 4 hr after an intraperitoneal injection of a chemical.

RESULTS

Effect of nicotinamide on DEN-induced ODC activity. The rats were fasted overnight and injected intraperitoneally with DEN. As reported previously [5], the ODC activity of the liver has increased 24-fold at 4 hr after the administration of DEN (Table 1). When nicotinamide was given to the rats simultaneously with DEN, the increase of ODC activity was inhibited by 80.4%. Though this result has not been reconciled with the report that ODC activity was induced by nicotinamide [2, 6], the discrepancy may be due to a difference in sensitivity of the animals used to the toxic dose of nicotinamide.

Effect of nicotinamide on DEN-induced tyrosine aminotransferase activity. Tyrosine aminotransferase in rat liver is a pyridoxal enzyme with a rapid turnover rate [7]. It is quite similar to ODC in that both enzymes are induced by a high protein diet [4], partial hepatectomy [8, 9] and the administration of DEN [5] (Table 1). In spite of the similarities of these two enzymes, tyrosine aminotransferase activity that was induced by DEN was not changed by simultaneous administration of nicotinamide (Table 1). This result indicates that the effect of nicotinamide on induction of ODC activity is a specific one and is not a mere inhibition of general protein synthesis.

Effects of poly(ADP-ribose)-polymerase inhibitors on DEN-induced ODC activity. Nicotinamide is known to be an inhibitor of poly(ADP-ribose)-polymerase, but at the same time it is a degradation product of NAD catalyzed by this enzyme. Large amounts of nicotinamide are known to be released from NAD by the extensive poly(ADP-ribos)ylation of various nuclear proteins [10] including automodification [11] triggered by DNA damage caused by ionic radiation or carcinogens [12]. Keeping this evidence in mind, we studied the effects of inhibitors of poly(ADP-ribose)-polymerase on induction of liver ODC activity. These inhibitors were intraperitoneally injected into fasted rats simultaneously with DEN (200 mg/kg body wt), and the ODC activity was assayed 4 hr after the treatment. As shown in Table 2, *o*-aminobenzamide inhibited the induction of ODC most powerfully (97.8%), followed by nicotinic acid (85.4%), nicotinamide (74.2%), *m*-aminobenzamide (61.2%), and *p*-aminobenzamide (30.8%). In contrast, the order of their abilities to inhibit poly(ADP-ribose)-polymerase *in vitro* was benzamide > *m*-aminobenzamide > nicotinamide > *o*-aminobenzamide > *p*-aminobenzamide [13].

Effects of niacin analogues on DEN-induced ODC activity. Niacin analogues are classified into two groups: one having niacin effects and the other having anti-niacin effects [1, 14–16]. Niacin and some of its analogues prevent pellagra in humans and animals, and in this sense have niacin effects. Agents with anti-niacin effects antagonize the niacin effect

Table 2. Inhibitory effects of poly(ADP-ribose)polymerase-inhibiting agents on induction of ODC activity by DEN

Chemicals	Liver ODC activity [nmol·min^{-1}·(g liver)$^{-1}$]
Control	0.43 ± 0.11 (4)
A	5.49 ± 0.41* (5)
DEN	9.10 ± 1.70* (4)
DEN + A	3.75 ± 0.44*,† (5)
Control	0.62 ± 0.13 (4)
B	3.72 ± 0.28*,‡ (5)
DEN	19.10 ± 3.96* (4)
DEN + B	13.40 ± 0.47* (5)
Control	0.62 ± 0.12 (4)
C	3.85 ± 0.28*,‡ (5)
DEN	19.10 ± 3.96 (4)
DEN + C	1.02 ± 0.18‡ (5)
Control	0.07 ± 0.01 (4)
D	0.20 ± 0.07*,‡ (5)
DEN	2.50 ± 0.03* (4)
DEN + D	0.78 ± 0.08*,‡ (5)
Control	0.44 ± 0.08 (4)
E	0.39 ± 0.08‡ (5)
DEN	9.42 ± 1.81* (4)
DEN + E	2.76 ± 0.69‡,§ (5)
Control	0.45 ± 0.08 (4)
F	0.19 ± 0.03‡,§ (5)
DEN	9.42 ± 1.81* (4)
DEN + F	1.75 ± 0.26‡,§ (5)

Male rats were fasted overnight. They received an intraperitoneal injection of DEN (200 mg/kg body wt) and/or a test compound 4 hr before being killed. Values are mean ± SEM with the number of animals given in parentheses. Chemicals and doses were as follows; A, *m*-aminobenzamide 0.75 mmol/kg body wt; B, *p*-aminobenzamide 3.75; C, *o*-aminobenzamide 3.75; D, benzamide 0.75; E, nicotinamide 7.5; and F, nicotinic acid 7.5.

* Significantly different from control group, $P < 0.001$.

†,‡ Significantly different from DEN group: † $P < 0.05$ and ‡ $P < 0.005$.

§ Significantly different from control groups, $P < 0.05$.

and thus induce pellagra by their administration, alone, to animals [1, 15, 16]. Keeping in mind these effects of niacin and its analogues, we were able to classify some of these compounds into four groups (Table 3, groups 1–4) by comparing their abilities to induce ODC activity and/or to inhibit DEN-induced ODC activity in rat liver. Numerals in parentheses following a compound are the percentage induction of ODC activity compared with that of DEN and the percentage inhibition of the ODC induction caused by DEN respectively. In group 1, quinolinic acid (−4 and 1.6) and 3-acetylpyridine (1.1 and 10.7) did not induce ODC activity by their administration alone. Nor did coadministration with DEN have any effects on the induction of ODC (Table 3, group 1). In group 2, though the compounds did not induce ODC activity; they inhibited ODC induction in various degrees when given simultaneously with DEN (Table 3, group 2). The following chemicals were categorized as group 2: nicotinic acid (−2.9 and 85.5), nicotinamide (−0.7 and 74.3), 3-hydroxymethylpyridine (−5 and 94.6), β-picoline (3.8 and 97.6), pyridine-3-aldehyde (−0.3 and 72.6) and ethylnicotinate (3.5 and 55.2). The six chemicals categorized in this group are all known to exhibit niacin effects [1, 14, 17, 18]. In group 3, tryptophan (95.7 and 12.6), *N'*-methylnicotinamide (47.5 and −1.5) and isonicotinic acid hydrazide (151.5 and −24.4) by administration alone induced ODC activity but did not inhibit the ODC activity induced by DEN (Table 3, group 3). In group 4, picolinic acid (60.3 and 93.3), 6-aminonicotinamide (41.5 and 84.0), pyridine-3-sulfonic acid (152.5 and 52.8) and thionicotinamide (29.7 and 75.9) induced ODC activity by themselves but inhibited the DEN-induced ODC activity of the liver in various degrees (Table 3, group 4). Three of them are known to have anti-niacin effects [19–22]. Picolinic acid is metabolically inert and has no niacin effect [23]. It is not known, however, whether this compound has an anti-niacin effect.

DISCUSSION

We have answered the three questions raised at the start of the present study, as described in the Introduction, as follows. (1) Nicotinamide inhibited the induction by DEN of ODC activity (Table 1). It also inhibited the ODC induction caused by partial hepatectomy or by a high protein diet (Y. Ninomiya and S. Yanagi, unpublished data). (2) The inhibitory effects on poly(ADP-ribose)-polymerase activity of nicotinamide and related compounds were not necessarily correlated with the inhibitory effects on the induction of ODC activity (Table 2). Therefore, though some reservation should be made on the final conclusion because the comparison was made between the experimental results done *in vivo* and *in vitro*, the results strengthen our consideration that the inhibition of ODC induction in liver by nicotinamide and its analogues is independent of the inhibition of poly(ADP-ribose)-polymerase. (3) Nicotinamide and related compounds were classified in Table 3 according to their effects on the ODC induction by DEN and are summarized in Table 4. A generalization which may be made from these data is that the agents which inhibit DEN-induced ODC activity in the liver have either niacin (group 2) or anti-niacin effects (group 4), with a few exceptions. The substances that have a niacin effect are known to be converted metabolically to niacin in the liver of animals [1, 14] and, thus, are precursors of NAD. Actually, the administration of pharmacological doses of these drugs augments the concentration of nicotinamide and NAD in the liver [17, 24]. Some of the drugs with an anti-niacin effect also are direct precursors of the synthesis of NAD-like compounds at the nucleotidyl transferase step and are converted to NAD-like compounds with or without NAD-like activity [21], and these substances are known, in some cases, to act as NAD competitors after they are converted to NAD analogues [25]. These reports led us to speculate that the observed inhibition of ODC induction by these agents with either niacin or anti-niacin effects may be accomplished on the NAD level. Therefore, we studied the direct effect of NAD

Table 3. Effects of chemicals with niacin-like action and chemicals with anti-niacin action on liver ODC activity in rats

Chemicals	Liver ODC activity [nmol·min^{-1}·(g liver)$^{-1}$]
Group 1. Chemicals which did not induce ODC or inhibit ODC induction by DEN	
Control	0.85 ± 0.09 (4)
A	0.51 ± 0.04*,† (5)
DEN	8.56 ± 1.00‡ (4)
DEN + A	8.44 ± 1.40‡ (5)
Control	0.33 ± 0.03 (4)
B	0.51 ± 0.34† (5)
DEN	16.40 ± 1.91‡ (4)
DEN + B	14.70 ± 4.40‡ (5)
Group 2. Chemicals which did not induce ODC but inhibited ODC induction by DEN	
Control	0.45 ± 0.08 (4)
C	0.19 ± 0.03*,† (5)
DEN	9.47 ± 1.90‡
DEN + C	1.75 ± 0.26†,‡ (5)
Control	0.45 ± 0.08 (4)
D	0.39 ± 0.08† (5)
DEN	9.47 ± 1.90‡ (4)
DEN + D	2.76 ± 0.69*,§ (5)
Control	0.76 ± 0.26 (4)
E	0.27 ± 0.03§ (5)
DEN	10.20 ± 2.82* (4)
DEN + E	1.27 ± 0.74§ (5)
Control	0.24 ± 0.33 (4)
F	0.75 ± 0.11† (5)
DEN	13.60 ± 2.20‡ (4)
DEN + F	0.56 ± 0.10† (5)
Control	0.44 ± 0.58 (4)
G	0.28 ± 0.05† (5)
DEN	16.40 ± 1.90‡ (4)
DEN + G	4.74 ± 1.89† (5)
Control	0.28 ± 0.08 (4)
H	0.67 ± 0.12*,† (5)
DEN	11.30 ± 1.48‡ (4)
DEN + H	5.21 ± 0.97‡,§ (5)
Group 3. Chemicals which induced ODC but did not inhibit ODC induction by DEN	
Control	0.30 ± 0.07 (4)
I	11.47 ± 2.06‡ (5)
DEN	11.96 ± 2.55‡ (4)
DEN + I	10.49 ± 5.00 (5)
Control	0.35 ± 0.10 (4)
J	5.67 ± 1.01‡ (5)
DEN	11.56 ± 3.04* (4)
DEN + J	11.71 ± 1.47‡ (5)
Control	0.35 ± 0.30 (4)
K	18.30 ± 3.26‡ (5)
DEN	12.20 ± 2.94* (4)
DEN + K	15.10 ± 0.27‡ (5)
Group 4. Chemicals which induced ODC and inhibited ODC induction by DEN	
Control	0.85 ± 0.09 (4)
L	5.50 ± 1.32* (5)
DEN	8.56 ± 1.00‡ (4)
DEN + L	1.37 ± 0.38† (5)
Control	0.51 ± 0.08 (4)
M	5.24 ± 0.10†,‡ (5)
DEN	11.90 ± 1.10‡ (4)
DEN + M	2.33 ± 0.69† (5)
Control	0.51 ± 0.08 (4)
N	17.80 ± 0.99‡,§ (5)
DEN	11.90 ± 1.10‡ (4)
DEN + N	5.98 ± 0.65†,‡ (5)
Control	0.08 ± 0.02 (4)
O	3.48 ± 0.89*,† (5)
DEN	11.53 ± 1.95‡ (4)
DEN + O	2.84 ± 0.65*,† (5)

Male rats were fasted overnight. Each received an intraperitoneal injection of DEN (200 mg/kg body wt) and/or a test compound 4 hr before being killed. Values are mean ± SEM with the number of animals given in parentheses. Chemicals and doses were as follows: A, quinolinic acid 1.8 mmol/kg body wt; B, 3-acetylpyridine 0.9; C, nicotinic acid 7.5; D, nicotinamide 7.5; E, 3-hydroxymethylpyridine 3.75; F, β-picoline 3.75; G, pyridine-3-aldehyde 7.5; H, ethylnicotinate 7.5; I, tryptophan 1.8; J, *N'*-methylnicotinamide 2,0; K, isonicotinic acid hydrazide 2.0; L, picolinic acid 1.8; M, 6-aminonicotinamide 2.0; N, pyridine-3-sulfonic acid 3.0; and O, thionicotinamide 3.0.

* Significantly different from control group, $P < 0.05$.
† Significantly different from DEN group, $P < 0.005$.
‡ Significantly different from control group, $P < 0.005$.
§ Significantly different from DEN group, $P < 0.05$.

on ODC activity. It was so toxic, however, that the rats became moribund by 4 hr after injection even of the lowest dose (0.36 mmol/kg body wt) in this study. The results obtained in repeated experiments were contradictory and confusing. There were two important problems about the administration of NAD, i.e. stability and permeability. In addition, Jacobson *et al.* [26] observed that a rapid degradation of NAD in $_3T_3$ cells was caused by carcinogenic *N*-nitroso compounds. Therefore, it is conceivable that NAD levels in the liver were not the same in the rats that received only an NAD injection and those that received both NAD and DEN. Therefore, we could not but conclude that the direct effect of NAD could not be determined under the present experimental conditions.

It is well known that an increase in ODC activity is observed when experimental animals are given

Table 4. Summary of the effects of niacin and its analogues on ODC activity in rat liver

Groups	Induction of ODC activity	Inhibition of ODC induction by DEN	Comments
1	−	−	
2	−	+	Chemicals with niacin effect
3	+	−	
4	+	+	Chemicals with anti-niacin effect with the exception of picolinic acid*

* The anti-niacin effect of picolinic acid has not been elucidated.

some stimulus which induces regeneration of hepatic cells, such as DEN administration [5] or partial hepatectomy [8]. Induction of ODC activity by the chemicals of groups 3 and 4, therefore, may be caused by regenerating stimuli after injury due to their hepatic toxicities.

Both quinolic acid in group 1 and tryptophan in group 3 are physiological precursors of nicotinamide [23] and thus have niacin effects. The fact that the synthesis of nicotinamide from tryptophan is low [27] may explain the observed exception of two chemicals from the behavior of the majority of the chemicals. The amount of nicotinamide synthesized from tryptophan and, probably, from quinolinic acid might not reach the effective level to inhibit the DEN-induced ODC activity. The previously observed [28] and presently confirmed fact that the administration of tryptophan alone induces liver ODC activity may be explained by some adverse effects of its metabolite(s) on the liver, whereas the metabolite(s) of quinolinic acid may not have adverse effects.

3-Acetylpyridine, which was classified as group 1, is relatively toxic, and 0.9 mmol/kg body weight was a maximum load to the rats. The toxic effect, however, may not be enough to cause a regenerating effect. Though this drug is known to be converted to an NAD-like analogue with NAD activity *in vitro* [29], perhaps an effective concentration was not attained in the liver in this experiment. In conclusion, the above results strongly indicate a relation between the ODC induction in the liver and NAD metabolism.

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