Pergamon

Biochemical Pharmacology, Vol. 50, No. 11, pp. 1933–1941, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)02091-8

METABOLISM OF 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) IN HUMAN PLACENTAL MICROSOMES

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(*Received* 31 *March* 1995; *accepted* 14 *July* 1995)

Abstract—The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) can be activated metabolically by cytochrome(s) P450 to DNA-damaging agents that result in the formation of tumors in various organs of several animal models. In the present study, 30-min incubations at 37° containing 5 mg/mL pooled human placental microsomes, 36 nmol NNK (including 2 μCi [5-^{3}H]NNK) and a 5 mM concentration of either NADH, NADPH, or both cofactors together resulted in the formation of 11.43 ± 0.32, 35.40 ± 4.64, and 44.05 ± 1.66 pmol 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)/mg protein/min (mean ± SD, N = 3), respectively. Similar experiments using 7, 9, and 11 mM NADH, NADPH, and both cofactors together in equimolar concentrations yielded results that suggest that NADH- and NADPH-dependent reductions of NNK are catalyzed by different enzymes. Computer simulations for the production of NNAL based on various kinetic models corroborated the conclusion drawn from the empirically derived data. In human placental microsomes, the $K_{m,app}$ and $V_{max,app}$ for the formation of NNAL were 1021.9 ± 251.5 μM and 4360.7 ± 991.7 pmol/mg protein/min, respectively. Inhibition of cytochrome P450-dependent activities by carbon monoxide and dicumarol (100 and 200 μM) resulted in an average increase of NNAL production of 40 and 56%, respectively, suggesting that P450-dependent biotransformation of NNK is occurring in the absence of inhibitors. Similarly, polyclonal goat IgG against rabbit P450 reductase resulted in a 12% increase in the production of NNAL when compared with control values. Thirty micromolar rutin, ethacrynic acid, cibacron blue 3GA, and iodoacetic acid, known inhibitors of certain human carbonyl reductase(s), incubated with placental microsomes containing an equimolar concentration of NNK, did not have a significant effect on the production of NNAL. These results establish that: (1) cytochromes P450 are likely involved in the metabolism of NNK by human placental microsomes, (2) metabolism of NNK to NNAL by human placental microsomes is catalyzed by an NADPH-dependent carbonyl reductase(s) and an NADH-dependent carbonyl reductase(s), and (3) reduction of NNK to NNAL is catalyzed by a placental microsomal carbonyl reductase(s) not previously described.

Key words: metabolism; placenta; nitrosamine; NNK; cytochromes P450; carbonyl reductase(s)

The role of tobacco and tobacco smoke as the agents responsible for various forms of cancer and other health-related effects in humans has been well documented [1–3]. Cigarette smoking, for example, has been linked to the incidence of cancers of the lung, larynx, esophagus, bladder, renal pelvis, and pancreas, while snuff dipping and tobacco chewing have been more closely associated with cancers of the oral cavity, buccal mucosa and gums [4, 5]. It has been estimated that presently in the U.S. 30% of the cancer mortality in men and women is associated with smoking and chewing of tobacco [6]. Moreover, human studies have demonstrated the adverse effects of cigarette smoking during pregnancy on the fetus. These effects include: low infant birth weight, prematurity, perinatal mortality [7], sudden infant death syndrome [8], and lower scores on tests for neurodevelopment levels [9]. Furthermore, other studies have associated some childhood cancers such as Wilm's Tumor, acute lymphoblastic leukemia, and non-Hodgkins lymphoma with passive exposure to tobacco smoke while *in utero* and/or early in life [10, 11]. Tobacco-related cancers have been attributed to a number of chemicals present in tobacco and tobacco smoke, which have been identified as carcinogens, cocarcinogens, procarcinogens, and tumor promoters in many different animal species [12, 13]. Among these chemicals, are a group known as the tobacco-specific nitrosamines of which NNK† is the most potent carcinogen, capable of inducing lung tumors in all animal species tested [14–16].

NNK is a tobacco-specific carcinogen that is normally formed from nitrosation of nicotine during tobacco processing and/or cigarette smoking [17]. The carcinogenicity of NNK has been attributed to a process of metabolic activation that yields reactive DNA methylating and pyridyloxobutylating agents [18–20]. This activation involves an α-hydroxylation at either the methylene or methyl carbon. The α-hydroxylation of the methylene carbon leads to the formation of a keto aldehyde and methyl diazohydroxide, whereas the α-hydroxylation of the methyl carbon yields formaldehyde and 4-(3-pyridyl)-4-oxobutyl diazohydroxide [18] (Fig. 1). The two metabolic activation pathways for NNK (α-hydroxylations) have been found to be mediated via different forms of cytochrome P450 in both human and animal studies [18, 19]. Carbonyl reductase has also been observed to metabolize NNK to NNAL, which can subsequently undergo α-hydroxylations via cytochromes P450 [18, 19] that lead to the formation of metabolites

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† Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; and NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

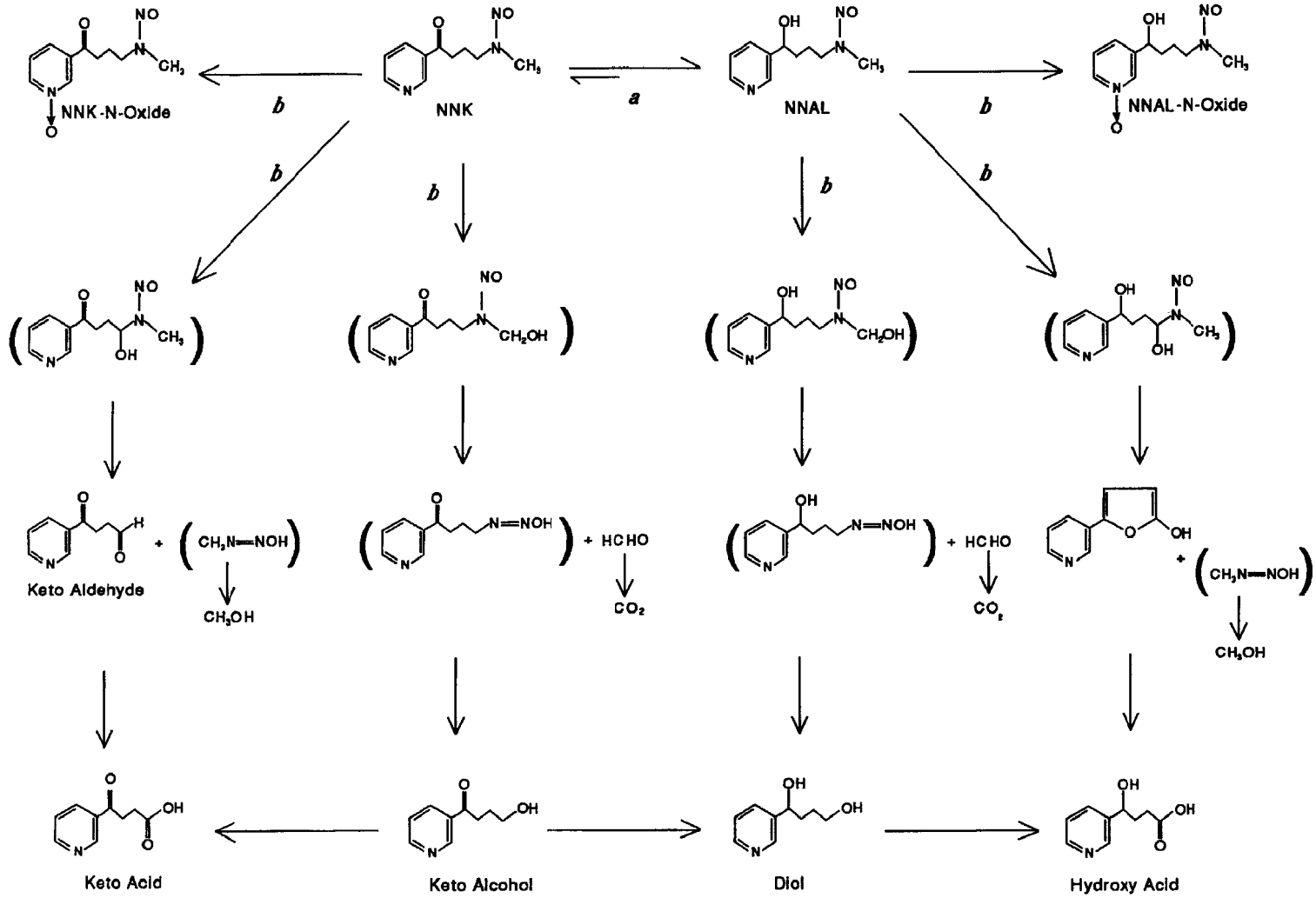

Fig. 1. Proposed metabolic pathways of NNK by hepatic and pulmonary microsomes. Pathway *a* is catalyzed by carbonyl reductase(s), while pathways *b* are cytochrome P450-mediated reactions. Structures in parentheses are hypothetical intermediates. These pathways are modified from Hecht *et al.* [21] and Jorquera *et al.* [22].

that can methylate or pyridyloxobutylate DNA in a manner similar to that which occurs with NNK metabolites.

While NNK has been found to induce lung tumors in all animal species tested [14–16], studies using Syrian Golden hamsters have also established NNK as a transplacental carcinogen [23]. Correa *et al.* [23] reported that 70% of the offspring that had been exposed to varying doses of NNK while *in utero* developed tumors in different organs, which included the respiratory tract, nasal cavity, adrenal glands, pancreas, and liver. In addition, two previous studies [24, 25] have demonstrated that NNK can be metabolized to NNAL in smokers and nonsmokers alike who are exposed to sidestream cigarette smoke, thus providing evidence for the link between exposure to environmental tobacco smoke and the risk of lung cancer. Because NNK has been identified as a transplacental carcinogen and because the human placenta is known to contain cytochromes P450 [26, 27], carbonyl reductases [28] such as aldehyde reductase (EC 1.1.1.2), aldose reductase (EC 1.1.1.21), and carbonyl reductase (prostaglandins 9-keto reductase), and DT-diaphorase (EC 1.6.99.2), the present work focuses on the metabolism of NNK by human placental microsomes.

MATERIALS AND METHODS

Chemicals

Unlabeled NNK and [5-^{3}H]NNK (2.29 Ci/mmol; purity > 96%) were purchased from Chemsyn Laboratories (Lenexa, KS). NNK metabolite standards were provided by Drs. Steven Hecht and Neil Trushin of the American Health Foundation (Valhalla, NY). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, $NADP^+$, NADPH, NADH, EDTA, magnesium chloride, rutin (quercetin 3β-D-rutinoside) trihydrate, ethacrynic acid ([2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid), methimazole (2-mercapto-1-methylimidazole), cibacron blue 3GA, iodoacetic acid, Trizma (tris[hydroxymethyl]aminomethane hydrochloride), potassium phosphate (dibasic; purity >98%) and barium hydroxide were purchased from the Sigma Chemical Co. (St. Louis, MO). Zinc sulfate and HPLC methanol were obtained from the Fisher Scientific Co. (Fair Lawn, NJ). Potassium phosphate (monobasic; purity >98%) was obtained from J. T. Baker (Phillipsburg, NJ). Carbon monoxide was purchased from Scott Specialty Gases (South Plainfield, NJ). Hydrochloric acid was obtained from EM Science (Gibbstown, NJ). The polyclonal goat IgG was against rabbit P450 reductase was purchased from Oxford Biomedical Research, Inc. (Oxford, MI).

Isolation of human placental microsomes

Placentae were obtained following repeat cesarean sections or vaginal deliveries from nonsmokers, who were not receiving any medications chronically. These placentae were immersed in ice-cold buffer (100 mM potassium phosphate, pH 7.4) as soon as possible in the delivery room. Tissues were transported to the laboratory within 15 min after recovery. Microsomal isolation was conducted according to the method of Sultatos and Murphy [29]. A portion of each placenta (120–160 g) was excised of connective tissue and homogenized in 3 vol. of buffer. The homogenate was filtered through cheesecloth and the volume noted. The homogenate was centrifuged for 10 min at 8360 *g* in a Sorvall RC5C (Wilmington, DE) centrifuge. The supernatant was collected and further ultracentrifuged at 100,000*g* for 1 hr in a Beckman L 7-55 (Palo Alto, CA). One milliliter of 100 mM potassium phosphate buffer was added to each microsomal pellet. A protein assay was conducted on each placenta using the method of Sedmak and Grossberg [30].

NNK metabolism catalyzed by human placental microsomes

Unless otherwise stated, the incubation mixture consisted of 100 mM potassium phosphate, pH 7.4, 5 mM glucose-6-phosphate, 4.56 U glucose-6-phosphate dehydrogenase, 1 mM $NADP^+$, 1 mM EDTA, 3 mM $MgCl_2$, 30 μM NNK (5 μCi [5-^{3}H]NNK), and 2 mg of microsomal protein in 1.2 mL. The reaction mixture was incubated at 37° for 10 min and terminated by the addition of 100 μL each of 25% zinc sulfate and saturated barium hydroxide. Chemical inhibitors and polyclonal goat IgG against rabbit cytochrome P450 reductase were dissolved in 100 mM potassium phosphate buffer. Incubation mixtures that consisted of 5 mg microsomal protein (pooled from four different placenta), an NADPH-generating system, and 30 μM NNK including 3 μCi [5-^{3}H]NNK were preincubated for 10 min with a 30 μM concentration of any of the following inhibitors: rutin, ethacrynic acid, cibacron blue, iodoacetic acid, and methimazole before incubating for 30 min. The reaction was initiated by the addition of substrate. A mixture of carbon monoxide and air (10%:90%) was bubbled through the microsome–buffer incubation mixture for 1 min prior to incubating and for 3 min beginning at min 15 of a 30-min incubation. The reaction was initiated by the addition of NNK and the NADPH-generating system. The NNK was dissolved in a volume of ethanol that was <1% of the total incubation volume. Polyclonal goat IgG against rabbit P450 reductase was added to human placental microsomes at 1:10, 1:50, and 1:100 dilutions and allowed to react for 30 min prior to initiating a 30-min incubation reaction with the addition of substrate.

Identification of metabolites

An HPLC method modified from Smith *et al.* [19] was used to identify metabolites. The HPLC system consisted of a Waters automated gradient controller, two Waters 501 pumps, a Waters U6K injector, a Waters 484 tunable UV detector and a C_{18} column (3.9 mm × 300 mm; Waters, Milford, MA). After precipitation of the protein and centrifugation of the samples, 200 μL of the incubation mixture supernatant was co-injected onto the system with 5 μL of unlabeled metabolite standards for identification. Separation of compounds was achieved with a linear gradient of 5% solvent A (methanol) and 95% solvent B (20 mM Tris–HCl buffer, pH 7.0) to 40% solvent A and 60% solvent B over a 50-min period. The radioactive peaks were identified by a Raytest Ramona 92 Radioactive Flow Detector (Wilmington, DE).

Computer model

A continuous system mathematical model of NNAL production from NNK, based on an Ordered BiBi kinetic mechanism (Fig. 2), was developed with the use of Advanced Continuous Simulation Language (Mitchell & Gauthier Associates, Concord, MA) and a Dell 320 SLi

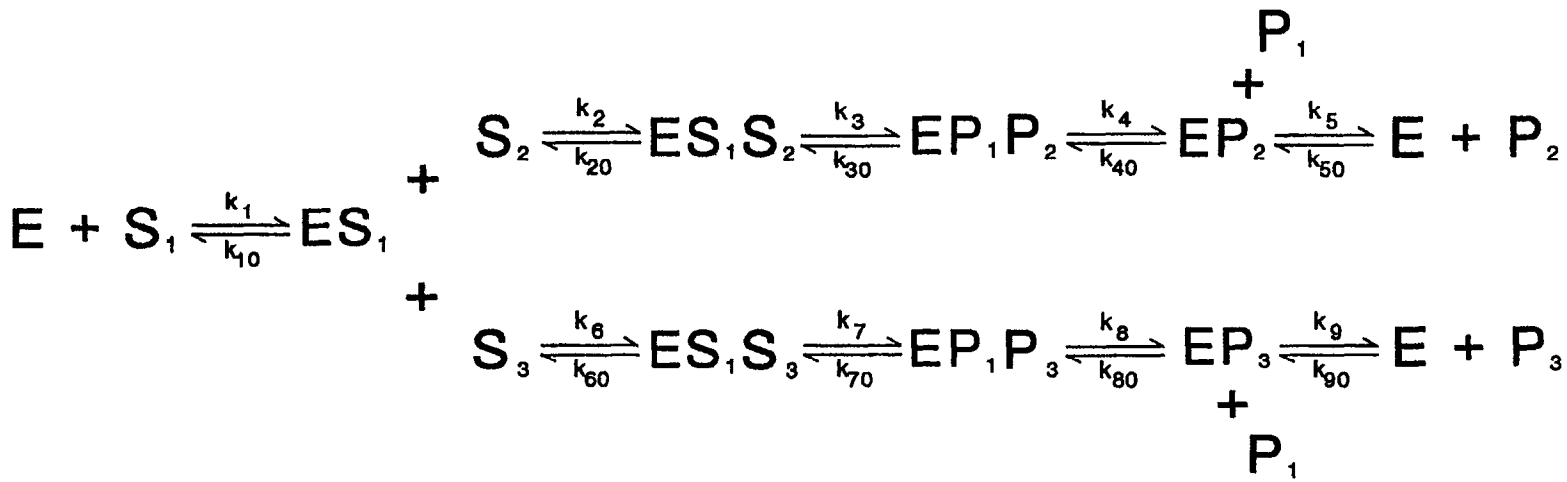

Fig. 2. Schematic diagram of NADH- and NADPH-dependent NNAL formation, assuming an Ordered BiBi kinetic mechanism, and assuming both cofactors compete for the same enzyme.

computer (Dell, Austin, TX). The differential mass-balance equations describing each phase of this reaction and the algebraic equations that account for the quantities of enzyme (E), NNK (S_1), NADPH (S_2), and NADH (S_3) involved in the reaction mechanism are outlined below. E_t and S_{1t}, S_{2t}, and S_{3t} represent the total enzyme and NNK, NADPH, and NADH concentrations, respectively; other symbols are defined in Fig. 2.

$$dES_1/dt = (k_1 \cdot E \cdot S_1) + (k_{20} \cdot ES_1S_2) + (k_{60} \cdot ES_1S_3) - (k_{10} \cdot ES_1) - (k_2 \cdot ES_1 \cdot ES_2) - (k_6 \cdot ES_1 \cdot S_3) \quad (1)$$

$$dES_1S_2/dt = (k_2 \cdot ES_1 \cdot S_2) + (k_3 \cdot EP_1P_2) - (k_{20}ES_1) - (k_3 \cdot ES_1S_2) \quad (2)$$

$$dEP_1P_2/dt = (k_3 \cdot ES_1S_2) + (k_{40} \cdot EP_2 \cdot P_1) - (k_{30} \cdot EP_1P_2) - (k_4 \cdot EP_1P_2) \quad (3)$$

$$dEP_2/dt = (k_4 \cdot EP_1P_2) + (k_{50} \cdot E \cdot P) - (K_{40} \cdot EP_2 \cdot P) - (k_5 \cdot EP_2) \quad (4)$$

$$dP_2/dt = (k_5 \cdot EP_2) - (k_{50} \cdot E \cdot P) \quad (5)$$

$$dES_1S_3/dt = (k_6 \cdot ES_1 \cdot S_3) + (k_{70} \cdot EP_1 \cdot P_3) - (k_{60} \cdot ES_1 \cdot S_3) - (k_7 \cdot ES_1S_3) \quad (6)$$

$$dEP_1P_3/dt = (k_7 \cdot ES_1S_3) + (k_{80} \cdot EP_3 \cdot P_1) - (k_{70} \cdot EP_1P_3) - (k_8 \cdot EP_1P_3) \quad (7)$$

$$dEP_3/dt = (k_8 \cdot EP_1P_3) + (k_{90} \cdot E \cdot P_3) - (k_{80} \cdot EP_3 \cdot P_1) - (k_9 \cdot EP_3) \quad (8)$$

$$dP_3/dt = (k_9 \cdot EP_3) - (k_{90} \cdot E \cdot P_3) \quad (9)$$

$$E = E_t - ES_1 - ES_1S_2 - EP_1P_2 - EP_2 - ES_1S_3 - EP_1P_3 - EP_3 \quad (10)$$

$$S_1 = S_{1t} - ES_1 - ES_1S_2 - EP_1P_2 - P_1 - ES_1S_3 - EP_1P_3 \quad (11)$$

$$S_2 = S_{2t} - ES_1S_2 - EP_1P_2 - EP_2 - P_2 \quad (12)$$

$$S_3 = S_{3t} - ES_1S_3 - EP_1P_3 - EP_3 - P_3 \quad (13)$$

Table 1 represents the values for the individual rate constants, obtained by optimization of the simulation to the empirically derived data.

Statistical analysis

The data were analyzed by Student's *t*-test or the appropriate analysis of variance followed by the Newman-Keuls multiple range test [31]. Addition of means ± SD in quadratures was made according to the method of Taylor [32].

Table 1. Constants utilized for the computer model of NNAL production from NNK based on an Ordered BiBi kinetic mechanism

Parameter	Value
E_t	0.025 μM^{-1}
k_1	80.000 $\mu M^{-1}hr^{-1}$
k_{10}	0.001 hr^{-1}
k_2	0.002 $\mu M^{-1}hr^{-1}$
k_{20}	0.001 hr^{-1}
k_3	9.000 hr^{-1}
k_{30}	0.001 hr^{-1}
k_4	18.000 hr^{-1}
k_{40}	0.001 $\mu M^{-1}hr^{-1}$
k_5	112.500 hr^{-1}
k_{50}	0.001 hr^{-1}
k_6	0.0025 hr^{-1}
k_{60}	0.001 hr^{-1}
k_7	4.000 hr^{-1}
k_{70}	0.001 hr^{-1}
k_8	5.000 hr^{-1}
k_{80}	0.001 hr^{-1}
k_9	2.500 hr^{-1}
k_{90}	0.001 hr^{-1}

RESULTS

NADPH- and NADH-dependent metabolism of NNK in human placental microsomes led primarily to the formation of NNAL (Fig. 3). The ketoalcohol of hydroxypyridyl butanone (Fig. 3) was also detected, but only consistently in two placentae. The identities of these two NNK metabolites were established by coelution with authentic unlabeled standards. Both NNK and NNAL have an entgegen (*E*) and a zuzammen (*Z*) structure and, therefore, elute as a major and a minor peak. The first peak represents the *E* form, while the second peak represents the *Z* form.

Production of NNAL from NNK required NADPH or NADH as a cofactor, although activity was much greater in the presence of NADPH at all concentrations of cofactors tested (Fig. 4). Inclusion of equimolar amounts of both cofactors in the incubations led to formation of NNAL that was nearly equal to the arithmetic sum of those incubations containing either cofactor alone (Fig. 4).

In the substrate concentration range of 50–2000 μM NNK, apparent Michaelis–Menten kinetics were observed for the formation of NNAL, using NADPH as a cofactor (Fig. 5). All experiments were carried out under conditions that resulted in linear reaction rates with respect to time and protein concentration (data not shown). Under the present experimental conditions, approximately 7% of the initial amount of NNK was metabolized in the placental microsomes. Regression analyses indicated a one-enzyme system (Fig. 5, Table 2), although the possibility of another enzyme(s) cannot be ruled out completely. Careful analysis of the kinetic studies in all placentae demonstrates what might be an inflection point (Fig. 5), possibly indicating the involvement of two or more NADPH-dependent enzymes in the metabolism of NNK.

Inhibition of P450-dependent monooxygenase activity by carbon monoxide or polyclonal goat IgG against cytochrome P450 reductase resulted in a significant increase in NNAL formation (Fig. 6). Similarly, inclusion of dicumarol in the incubations led to increased formation of NNAL (Fig. 6).

Incubations containing 30 μM methimazole, an inhibitor of the flavin-monooxygenase system, had no effect on the metabolism of NNK (Fig. 6). Likewise, inhibitors of aldehyde reductase, aldose reductase, and carbonyl reductase did not have an effect on the production of NNAL (Fig. 6).

DISCUSSION

The metabolism of NNK to the various DNA-damaging agents has been demonstrated to be mediated via cytochromes P450 in both human and animal tissues [18, 19]. Smith *et al.* [19] reported that in mouse lung microsomes, for example, CYP1A1, CYP2B1, and CYP2B2 led to the formation of ketoalcohol (Fig. 1). In twelve forms of cytochromes P450 expressed in human hepatoma G2 cells, however, CYP1A2 had the highest activity in catalyzing the formation of ketoalcohol (α-hydroxylation product) at all NNK concentrations used.

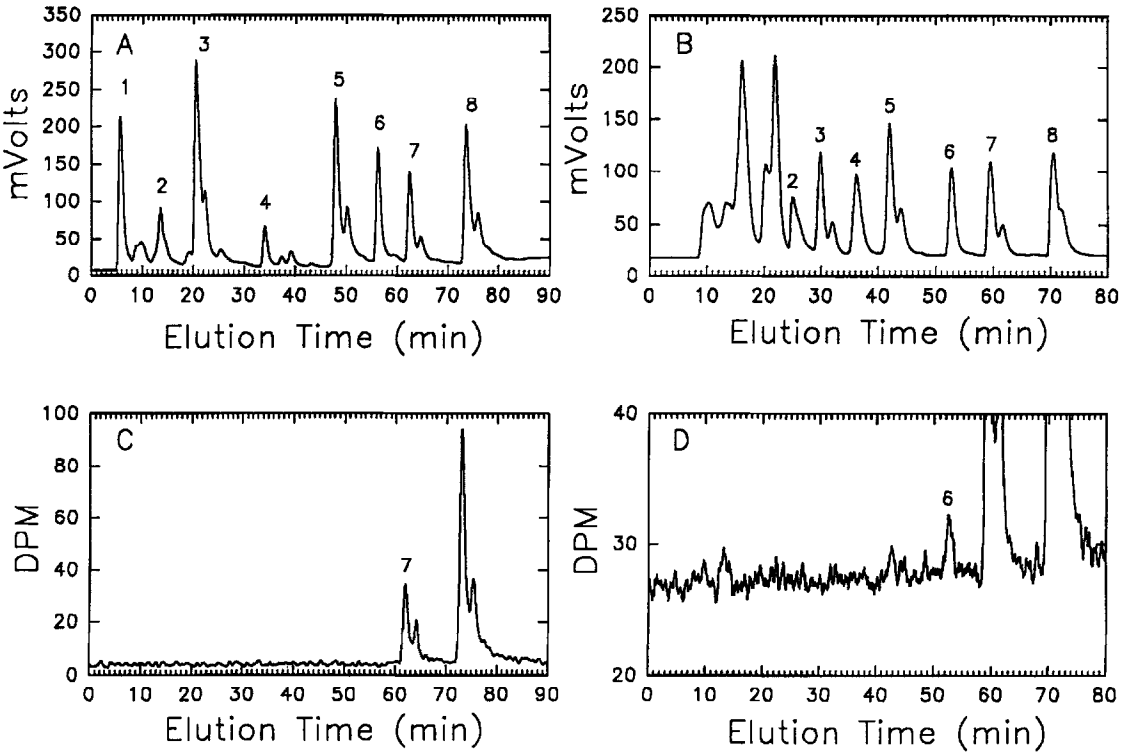

Fig. 3. Identification of NNAL and ketoalcohol using NNK metabolite standards. Panels A and B represent chromatograms of NNK and metabolite standards, while panels C and D represent chromatograms of the products of NN placental incubations. Below is a list of NNK and its metabolites in order of elution:

(1) 4-hydroxy-4-(3-pyridyl)-butyric acid (hydroxy acid)
(2) 4-oxo-4-(3-pyridyl)-butyric acid (keto acid)
(3) 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol (NNAL-*N*-oxide)
(4) 4-hydroxy-1-(3-pyridl)-1-butanol (diol)
(5) 4-(methylnitrosamino)-1-(pyridyl-*N*-oxide)-1-butanone (NNK-*N*-oxide)
(6) 4-hydroxy-1-(3-pyridyl)-1-butanone (keto alcohol)
(7) 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)
(8) 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

CYP2A6, CYP2B7, CYP2E1, CYP2F1, and CYP3A5 also led to the production of ketoalcohol, but at lower rates. CYP2C8 catalyzed the formation of NNK-*N*-oxide (pyridine *N*-oxidation product) at all NNK concentrations, but at a very low rate. In addition, the involvement of CYP1A2 in the formation of ketoalcohol has been demonstrated in human liver microsomes [19].

In the present study, the metabolism of NNK in human placental microsomes was observed to follow the reductive pathway in all placentae tested, whereas cytochrome P450-dependent metabolism was only directly observed in two placentae. The formation of NNAL (reductive pathway) was observed whenever NNK was incubated with placental microsomes in the presence of either or both cofactors NADPH or NADH. NNAL production likely occurs via two or more enzymes since incubations containing equimolar concentrations of each cofactor produced a level of NNAL that was additive rather than less than additive when compared with incubations containing cofactors separately. Further support for a two- or multiple-enzyme hypothesis was obtained from a continuous system mathematical model of NNAL production from NNK, based on an Ordered BiBi kinetic mechanism. These results indicate that in a system where both cofactors compete for the same enzyme, the production of NNAL should lie between that produced in those incubations containing NADPH alone and those containing NADH alone and would not equal the sum of each cofactor, as the experimentally derived data show (Fig. 4). Other computer simulations based on a Random BiBi kinetic mechanism have yielded similar results (data not shown).

The kinetic parameters for the production of NNAL using NADPH as a cofactor in five different human placental samples show a remarkably high $V_{max,\,app}/K_{m,\,app}$ ratio (Table 2), unlike previous studies using human lung microsomes [19], in which the $V_{max,\,app}/K_{m,\,app}$ was equal to 0.6. In the present work, the average $V_{max,\,app}/K_{m,\,app}$ ratio was 7-fold higher than observed in human lung microsomes (Table 2), suggesting that NNK may be a more suitable substrate for carbonyl reductase(s) in the human placentae than for that in human lung. In addition, the high activity for NNAL production as evidenced by the high apparent V_{max} indicates that an average human placenta of approximately 600 g has a theoretical potential to produce close to 3 μmol NNAL/min

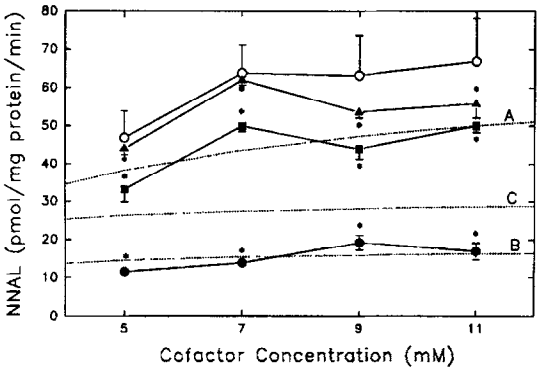

Fig. 4. NADH- and NADPH-dependent production of NNAL by human placental microsomes. Each data point represents the mean ± SD of 3 determinations using pooled microsomes from four placentae. Key: (●) NADH alone; (■) NADPH alone; (▲) empirical data using equimolar concentrations of NADH and NADPH; and (○) the sum of NADH and NADPH activities. Line A represents a computer simulation of NADPH alone, while line B represents a computer simulation of NADH alone. Line C represents a computer simulation of equimolar concentrations of NADH and NADPH assuming that they both serve as cofactors for the same enzyme. An asterisk (*) indicates significance from each other ($P < 0.05$) as determined by a two-way analysis of variance followed by the Newman-Keuls multiple range test.

(Table 2). This is within the range of nitrosamines known to cause lung tumors in rats when administered subcutaneously [33].

Although the metabolism of NNK to other metabolites was not detected clearly with the exception of ketoalcohol in two placentae, incubations in the presence of carbon monoxide, polyclonal goat IgG against cytochrome P450 reductase, and dicumarol provided indirect evidence of cytochrome P450 involvement. These results suggest that (a) cytochrome P450-dependent formation of NNK metabolites was inhibited, leading to increased concentration of NNK available to undergo the reductive pathway, and/or (b) subsequent metabolism of NNAL to DNA-damaging agents was inhibited, also leading to increased levels of NNAL. Surprisingly, however, in those incubations where ketoalcohol was detected and dicumarol was used, the levels of ketoalcohol were unaffected by the presence of dicumarol (data not shown). One possible explanation for this outcome may be that the formation of ketoalcohol was already at maximum velocity such that competitive inhibition of NNK metabolism at the dicumarol concentrations used did not result in a detectable difference in the levels of ketoalcohol.

Since the major metabolite of NNK in human placental microsomes was NNAL, inhibitors of several human carbonyl reductases were used. The concentrations of the inhibitors utilized were either comparable or considerably higher than those previously used by other investigators [34] such that any inhibitory effects would have been clearly noted. Because the levels of NNAL did not decrease in the presence of these inhibitors (Fig. 6), the enzyme(s) involved in the production of NNAL is not aldehyde reductase, aldose reductase, or carbonyl reductase. In addition, the results of dicumarol (also known to inhibit DT-diaphorase) suggest that DT-diaphorase is also not involved in the production of NNAL from NNK since the production of NNAL increased rather than decreased in those incubations containing dicumarol. Thus, the enzyme(s) mediating the formation of NNAL does not appear to be a reductase previously described.

That NNK can be metabolized by human placenta *in vitro* is of great toxicological significance for a number of reasons that may elucidate some of the *in vivo* risks

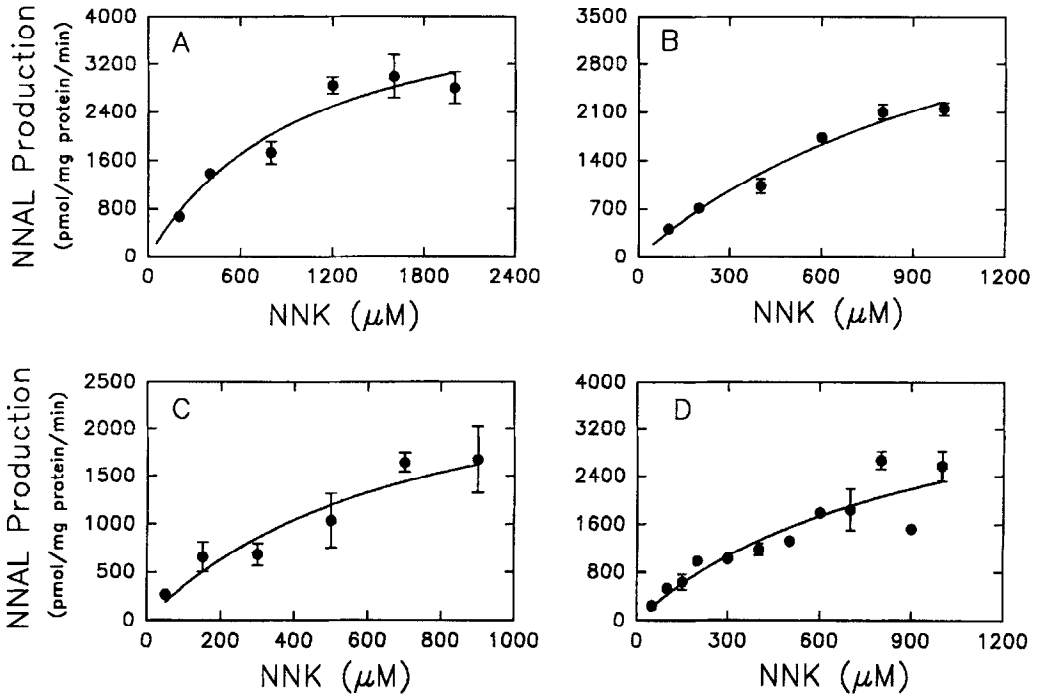

Fig. 5. Substrate dependency for the metabolism of NNK in microsomes isolated from four different human placentae (A–D). Each point represents the mean ± SD of three replicates.

Table 2. Apparent K_m, apparent V_{max}, $V_{max,app}/K_{m,app}$ ratios, and placental capacity for the production of NNAL from NNK

Placenta	$K_{m,app}$ (μM)	$V_{max,app}$ (pmol/mg protein/min)	$V_{max,app}/K_{m,app}$	Placental capacity for NNAL production (nmol/g tissue)
A	1038.9	4648.9	4.5	3.2
B	1340.0	5270.0	3.9	4.8
C	727.5	2948.5	4.1	5.3
D	981.2	4575.5	4.7	5.7
Mean ± SD	1021.9 ± 251.5	4360.7 ± 991.7	4.3 ± 0.4	4.8 ± 1.1

Values were determined in microsomes isolated from four different human placentae.

for the fetus associated with maternal smoking and/or exposure to cigarette smoke during pregnancy. First, the *in vivo* absorption of NNK and its subsequent metabolism to NNAL has been shown to occur in humans exposed to sidestream cigarette smoke [24, 25]. In addition, the transplacental crossing of nicotine and cotinine has been reported to occur in humans as evidenced by the content of these compounds in the hair of newborns whose mothers smoked or were exposed to secondhand smoke during pregnancy [35]. Since NNK is the oxidative product of nicotine [33] and is therefore a closely related molecule, it may be possible that NNK can also cross the placenta. Furthermore, NNK has been found to be a transplacental carcinogen in Syrian golden hamsters [23]. This carcinogenicity is a result of metabolic activation of NNK, which is mediated via cytochromes P450. This is important because human placenta also contains cytochrome P450 [27], particularly cigarette smoke-inducible CYP1A1 [36], which has been shown to activate NNK in mouse lung microsomes [18]. Consequently, mothers who actively smoke during pregnancy may introduce the combined effects of CYP1A1 induction (and other inducible cytochromes P450 capable of activating NNK or NNAL) with the enhanced metabolism of these compounds, potentially leading to the formation of higher levels of DNA-damaging agents.

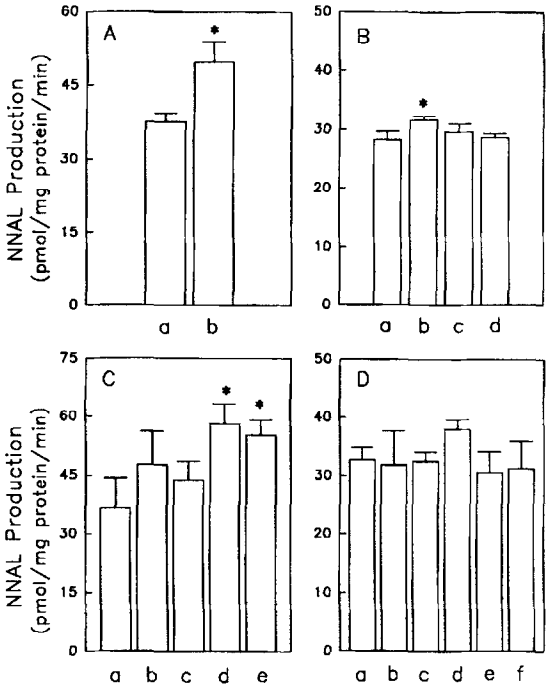

Fig. 6. Effects of different inhibitors on the production of NNAL from NNK. Panel A demonstrates the effects of carbon monoxide on the production of NNAL in microsomes pooled from three different human placentae (bar *b*) in comparison to controls (bar *a*). Each bar represents the mean ± SD of 5 replicates. Panel B demonstrates the effects of varied dilutions, 0 (bar *a*), 1:10 (bar *b*), 1:50 (bar *c*), and 1:100 (bar *d*) of polyclonal goat IgG against cytochrome P450 reductase on the productin of NNAL from NNK in microsomes isolated from three different placentae. Each bar represents the mean ± SD of three replicates. Panel C demonstrates the production of NNAL in the presence of 0 μM (bar *a*), 10 μM (bar *b*), 50 μM (bar *c*), 100 μM (bar *d*), and 200 μM (bar *e*) dicumarol in microsomes pooled from four different placentae. Each bar represents the mean ± SD of three replicates. Panel D demonstrates the production of NNAL in the presence of either 30 μM rutin (bar *b*), ethacrynic acid (bar *c*), cibacron blue 3GA (bar *d*), iodoacetic acid (bar *e*), or methimazole (bar *f*) in microsomes pooled from three different placentae. Controls are represented by bar *a*. Each bar represents the mean ± SD of three replicates. In all panels, an asterisk (*) indicates significance ($P < 0.05$) from control.

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