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Metabolism and Disposition Kinetics of Nicotine

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***Abstract*——Nicotine is of importance as the addictive chemical in tobacco, pharmacotherapy for smoking cessation, a potential medication for several diseases, and a useful probe drug for phenotyping cytochrome P450 2A6 (CYP2A6). We review current knowledge about the metabolism and disposition kinetics of nicotine, some other naturally occurring tobacco alkaloids, and nicotine analogs that are under development as potential therapeutic agents. The focus is on studies in humans, but animal data are mentioned when relevant to the interpretation of human data. The pathways of nicotine metabolism are described in detail. Absorption, distribution, metabolism, and excretion of nicotine and related compounds are reviewed. Enzymes involved in nicotine metabolism including cytochrome P450 enzymes, aldehyde oxidase, flavin-containing monooxygenase 3, amine *N*-methyltransferase, and UDP-glucuronosyltransferases are represented, as well as factors affecting metabolism, such as genetic variations in metabolic enzymes, effects of diet, age, gender, pregnancy, liver and kidney diseases, and racial and ethnic differences. Also effects of smoking and various inhibitors and inducers, including oral contraceptives, on nicotine metabolism are discussed. Due to the significance of the CYP2A6 enzyme in nicotine clearance, special emphasis is given to the effects and population distributions of *CYP2A6* alleles and the regulation of CYP2A6 enzyme.**

I. Introduction

Smoking has enormous negative health consequences worldwide, and the use of tobacco is still rising globally (Mackay and Eriksen, 2002). Nicotine is not a direct cause of most tobacco-related diseases, but it is highly addictive (Benowitz, 1999; Balfour, 2002). The addictiveness of nicotine is the cause of the continuing use of tobacco products, which in turn results in exposure to the diverse array of carcinogens and other bioactive compounds in tobacco, making tobacco use the leading cause of premature deaths in developed countries (Peto et al., 1992; Hecht, 2003). Tobacco is the single greatest preventable cause of death due to cancer. The vast majority of smokers want to quit, but due to nicotine addiction only a few percent of smokers quit successfully each year (CDC, 1993; USDHHS, 2000).

Nicotine medications are widely used as nicotine replacement therapies to assist smoking cessation and more recently have been proposed for use concurrently with smoking as part of a risk reduction strategy. Nicotine has also been studied as an experimental therapy for Parkinson's disease, Alzheimer's disease, and ulcerative colitis (Jani and Regueiro, 2002; Quik and Kulak, 2002; Sabbagh et al., 2002). Besides having importance as an addictive substance and a pharmaceutical, nicotine is of consequence as a specific substrate for cytochrome P450 2A6 (CYP2A6), thus facilitating the study of this enzyme in humans (Raunio et al., 2001).

We review current knowledge about the pharmacokinetics and metabolism of nicotine, some other naturally occurring tobacco alkaloids, and nicotine analogs that are under development as potential therapeutic agents. The focus is on studies in humans, but animal data are mentioned when relevant to the interpretation of human data. The pharmacokinetics of tobacco-specific nitrosamines, which are formed from tobacco alkaloids, is beyond the scope of this review. Their metabolism has been extensively reviewed elsewhere (Hecht, 1998).

II. Nicotine and Related Alkaloids in Tobacco Products

Nicotine (Fig. 1) is a natural ingredient in tobacco leaves where it acts as a botanical insecticide (Soloway, 1976; Tomizawa and Casida, 2003). It is the principal tobacco alkaloid occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content (Schmeltz and Hoffmann, 1977; Benowitz et al., 1983a). Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half of the nicotine concentration of the cigarette tobacco (Tilashalski et al., 1994; Lu and Ralapati, 1998; Jacob et al., 1999). An average tobacco rod contains 10 to 14 mg of nicotine (Kozlowski et al., 1998), and on average about 1 to 1.5 mg of nicotine is absorbed systemically during smoking (Benowitz and Jacob, 1984). The nicotine in tobacco is largely the levorotary (*S*)-isomer, only 0.1 to 0.6% of total nicotine content is (*R*)-nicotine (Armstrong et al., 1998). Chemical reagents and pharmaceutical formulations of (*S*)-nicotine have a similar content of (*R*)-nicotine (0.1–1.2%) as an impurity since plant-derived nicotine is used for their manufacture (Armstrong et al., 1998). The (*R*)-nicotine content of tobacco smoke is higher. Up to 10% of nicotine in smoke has been reported to be (*R*)-isomer, presumably resulting from racemization occurring during combustion (Klus and Kuhn, 1977; Pool et al., 1985).

In most tobacco strains, nornicotine and anatabine are the most abundant of the minor alkaloids, followed by anabasine (Fig. 1) (Schmeltz and Hoffmann, 1977; Saitoh et al., 1985). This order of abundance is the same in ciga-

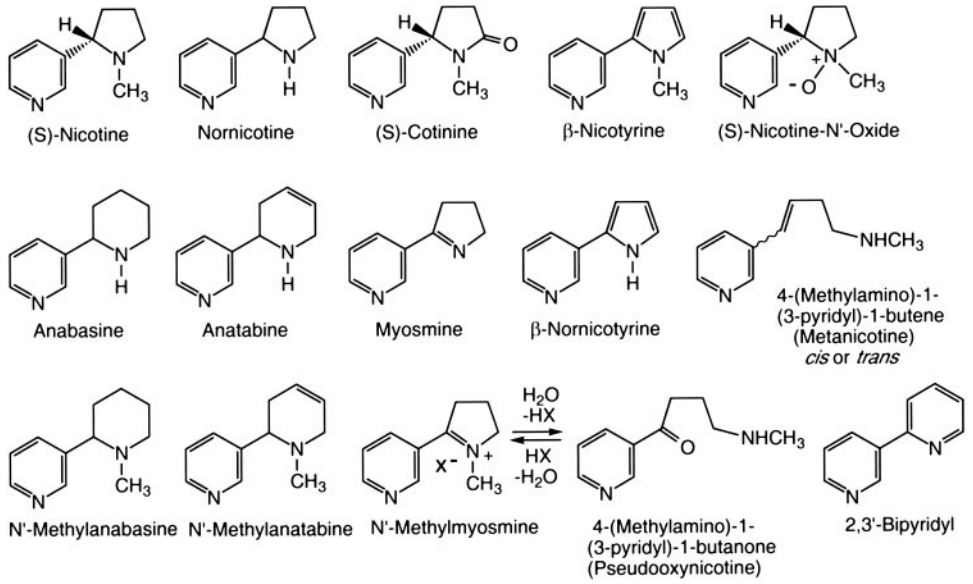

FIG. 1. Structures of tobacco alkaloids. Reprinted from Benowitz and Jacob, 1998 with permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

rette tobacco and oral snuff, chewing, pipe, and cigar tobacco (Jacob et al., 1999). However, nornicotine levels are highest in cigar tobacco, anatabine levels are lowest in chewing tobacco and oral snuff, and anabasine levels are lowest in chewing tobacco (Jacob et al., 1999). Small amounts of the *N*′-methyl derivatives of anabasine and anatabine are found in tobacco and tobacco smoke. Several of the minor alkaloids are thought to arise by bacterial action or oxidation during tobacco processing rather than by biosynthetic processes in the living plant (Leete, 1983). These include myosmine, *N*′-methylmyosmine, cotinine, nicotyrine, nornicotyrine, nicotine *N*′-oxide, 2,3′-bipyridyl, and metanicotine (Fig. 1) (Schmeltz and Hoffmann, 1977). Myosmine has been thought to be tobacco-specific but recent studies show that myosmine is found in a variety of foods including nuts, cereals, milk, and potatoes (Zwickenpflug et al., 1998; Tyroller et al., 2002). Also nicotine is found in low levels in vegetables such as potatoes, tomatoes, and eggplants (Domino et al., 1993; Siegmund et al., 1999).

N-Nitroso derivatives of tobacco alkaloids arise by the action of nitrous acid on nicotine, nornicotine, anabasine, and anatabine. These nitroso compounds are important because some are carcinogenic (Hecht and Hoffmann, 1989). Eight tobacco-specific nitrosamines have been identified (Hecht, 2003). *N*′-Nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK[1]), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) are the most carcinogenic. They all are derived from nicotine; *N*′-nitrosonornicotine is also derived from nornicotine (Hecht, 1998). Unlike nicotine, the minor alkaloids anatabine, nornicotine, and anabasine and their *N*-nitroso derivatives are present to a substantial degree as the (*R*)-isomer (about 16, 20, and 42%, respectively) in tobacco products (Armstrong et al., 1999; Carmella et al., 2000).

Of the minor alkaloids that have been studied, nornicotine, metanicotine, and anabasine have been shown to have significant pharmacologic activity (Clark et al., 1965). Qualitatively, their actions are similar to those of nicotine, but they are generally less potent, the relative potency depending upon the test system. Anabasine administered orally or sublingually has been reported to aid smoking cessation and to have cardiovascular effects (Nasirov et al., 1978). Ingestion of anabasine-containing *Nicotiana glauca* leaves has been reported to lead to nicotine-like poisoning and death by respiratory paralysis (Castorena et al., 1987; Mellick et al., 1999; Sims et al., 1999; Mizrachi et al., 2000; Steenkamp et al., 2002). Cotinine, the primary metabolite of nicotine in humans, has little or no effects on cognitive performance and no cardiovascular effects in humans, but has been reported to modify symptoms of nicotine withdrawal (Benowitz et al., 1983b; Keenan et al., 1994; Hatsukami et al., 1997, 1998a,b; Herzig et al., 1998; Zevin et al., 2000b). *Trans*-3′-hydroxycotinine, the main metabolite of cotinine, has no cardiovascular effects (Scherer et al., 1988; Benowitz

[1]Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NRT, nicotine replacement therapy; FMO, flavin-containing monooxygenase; UGT, uridine diphosphate-glucuronosyltransferase; P450, cytochrome P450; PXR, pregnane X receptor; CAR, constitutive androstane receptor; OCTN1, organic cation transporter number 1; ABT-418, (*S*)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole hydrochloride; ABT-089, 2-methyl-3-(2-(*S*)-pyrrolidinylmethoxy) pyridine dihydrochloride; ABT-594, (*R*)-5-(2-azetidinylmethoxy)-2-chloropyridine; SIB-1765F, [±]-5-ethynyl-3-(1-methyl-2-pyrrolidinyl) pyridine fumarate; SIB-1508Y, [*S*]-[−]-5-ethynyl-3-(1-methyl-2-pyrrolidinyl) pyridine maleate; RJR-2403, (*E*)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine; GTS-21 or DMXB, 3′-(2,4-dimethoxybenzylidine)-anabaseine; SIB-1663, [±]-7-methoxy-2,3,3*a*,4,5,9*b*-hexahydro-1*H*-pyrrolo-[3,2*h*]-isoquinoline; A-85380, 3-[2(*S*)-2-azetidinyl-methoxy]pyridine.

and Jacob, 2001). To our knowledge, no studies of the pharmacologic effects of any of the other minor alkaloids in humans have been reported.

III. Absorption of Nicotine from Tobacco Smoke and Nicotine Medications

Nicotine is distilled from burning tobacco and is carried proximally on tar droplets (also called particulate matter) which are inhaled. Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a pK_a of 8.0 (Fowler, 1954). In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. The pH of smoke from flue-cured tobaccos, found in most cigarettes, is acidic (pH 5.5–6.0) (Sensabaugh and Cundiff, 1967; Brunnemann and Hoffmann, 1974). At this pH, nicotine is primarily ionized. As a consequence, there is little buccal absorption of nicotine from flue-cured tobacco smoke, even when it is held in the mouth (Gori et al., 1986). Smoke from air-cured tobaccos, the predominant tobacco used in pipes, cigars, and some European cigarettes, is more alkaline (pH 6.5 or higher), and considerable nicotine is unionized (Sensabaugh and Cundiff, 1967). Smoke from these products is well absorbed through the mouth (Armitage et al., 1978). It has recently been proposed that the pH of cigarette smoke particulate matter is higher than previously thought, and thus, a larger portion of nicotine would be in the unionized form, facilitating rapid pulmonary absorption (Pankow, 2001). The effective pH values for particulate matter in various brands of cigarettes were measured to span a range of 6.0 to 7.8 (Pankow et al., 2003).

When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed. Blood concentrations of nicotine rise quickly during and peak at the completion of cigarette smoking (Fig. 2). The rapid absorption of nicotine from cigarette smoke through the lungs, presumably because of the huge surface area of the alveoli and small airways, and dissolution of nicotine in the fluid of pH 7.4 in the human lung facilitates transfer across membranes. On average, about 1 mg (range 0.3–2 mg) of nicotine is absorbed systemically during smoking (Benowitz and Jacob, 1984; Gori and Lynch, 1985). About 80 to 90% of inhaled nicotine is absorbed during smoking as assessed using ^{14}C-nicotine (Armitage et al., 1975). The efficacy of absorption of nicotine from environmental smoke in nonsmoking women has been measured to be 60 to 80% (Iwase et al., 1991). After a puff, high levels of nicotine reach the brain in 10 to 20 s, faster than with intravenous administration, producing rapid behavioral reinforcement through the activation of the dopaminergic reward system (Benowitz, 1990, 1996b). The rapidity of rise in nicotine levels permits the smoker to titrate the level of nicotine and related effects during smoking and makes smoking the most reinforcing and dependence-producing form of nicotine administration (Benowitz, 1990; Henningfield and Keenan, 1993).

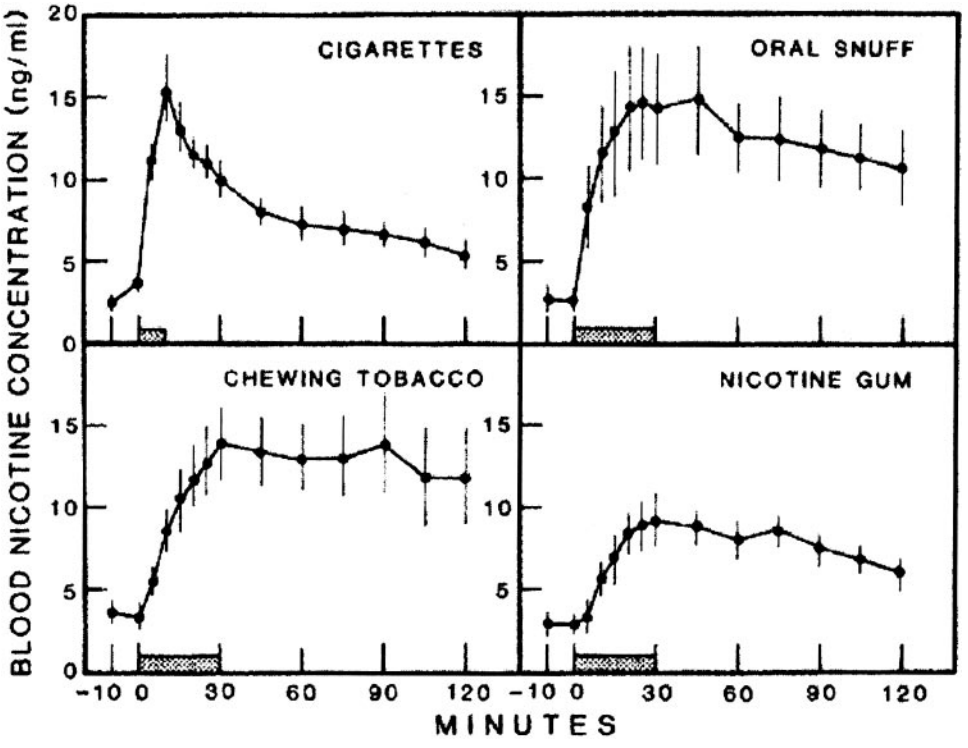

FIG. 2. Blood nicotine concentrations during and after cigarette smoking for 9 min, oral snuff (2.5 g), chewing tobacco (average 7.9 g), and nicotine gum (two 2-mg pieces). Average values for 10 subjects (±S.E.M.). Reprinted from Benowitz et al., 1988 with permission from American Society for Clinical Pharmacology and Therapeutics.

The process of cigarette smoking is complex, and as mentioned above, the smoker can manipulate the dose of nicotine and nicotine brain levels on a puff by puff basis. The intake of nicotine during smoking depends on the puff volume, the depth of inhalation, the extent of dilution with room air, the rate of puffing, and the intensity of puffing. For this reason, the machine-determined nicotine yields of cigarettes (U.S. Federal Trade Commission, FTC yields) cannot be used to estimate the dose of nicotine by a smoker (Russell et al., 1980; Benowitz et al., 1983a; Jarvis et al., 2001). In general, cigarette smokers switching from a higher to a lower-yield cigarette will compensate, i.e., will change the smoking pattern to gain more nicotine (USDHHS, 2001).

Chewing tobacco and snuff are buffered to alkaline pH to facilitate absorption of nicotine through oral mucosa (Benowitz, 1999). Although the absorption through cell membranes is rapid for these more alkaline tobacco products, the rise in the brain nicotine level is slower than with smoking. Concentrations of nicotine in the blood rise gradually with the use of smokeless tobacco and plateau at about 30 min with levels persisting and declining only slowly over 2 h or more (Fig. 2) (Benowitz et al., 1988).

The various formulations of nicotine replacement therapy (NRT), such as nicotine gum, transdermal patch, nasal spray, inhaler, sublingual tablets, and lozenges, are buffered to alkaline pH to facilitate the absorption of nicotine through cell membranes. Absorption of nicotine from all NRTs is slower and the increase in nicotine blood levels more gradual than from smoking (Table 1). This slow increase in blood and especially brain levels results in low abuse liability of NRTs (Henningfield and Keenan, 1993; West et al., 2000). Only nasal spray provides a rapid delivery of nicotine that is closer to the rate of nicotine delivery achieved with smoking (Sutherland et al., 1992; Gourlay and Benowitz, 1997; Guthrie et al., 1999). The absolute dose of nicotine absorbed systemically from nicotine gum is much less than the nicotine content of the gum, in part, because considerable nicotine is swallowed with subsequent first-pass metabolism (Benowitz et al., 1987). Some nicotine is also retained in chewed gum. A portion of the nicotine dose is swallowed and subjected to first-

TABLE 1
Nicotine absorption pharmacokinetics of different forms of nicotine administration in single doses

Type of Nicotine Administration[a]	C_{max}[b]	T_{max}[b,c]	Bioavailability	References
	ng/ml	*min*	*%*	
Smoking (one cigarette, 5 min) (~2 mg/cigarette[d])	15–30 (venous) 20–60 (arterial)	5–8 (venous) 3–5 (arterial)	80–90 (of inhaled nicotine)	Armitage et al., 1975; Russell et al., 1983; Benowitz et al., 1988; Henningfield et al., 1993; Rose et al., 1999; Lunell et al., 2000
Intravenous ~5.1 mg (60 μg/kg, 30 min)	30 (venous) 50 (arterial)	30 (venous) 30 (arterial)	100	Gourlay and Benowitz, 1997
Nasal spray 1 mg	5–8 (venous) 10–15 (arterial)	11–18 (venous) 4–6 (arterial)	60–80	Johansson et al., 1991; Gourlay and Benowitz, 1997; Guthrie et al., 1999
Gum (30 min, total dose in gum)				Russell et al., 1980b, 1983
2 mg	6–9	30	78	Benowitz et al., 1988
4 mg	10–17	30	55	Stevens, 1994
Inhaler 4 mg released (one 10 mg cartridge, 20 min)	8.1	30	51–56	Molander et al., 1996; Schneider et al., 2001
Lozenge (20–30 min)				Choi et al., 2003
2 mg	4.4	60	50	
4 mg	10.8	66	79	
Sublingual tablet 2 mg (20–30 min)	3.8	~60	65	Molander and Lunell, 2001
Tooth patch 2 mg	~3.2	~120		Slamowitz et al., 2000
Transdermal patch (labeled dose)				Benowitz et al., 1991a, 1992
15 mg/16 h (Nicotrol)	11–14	6–9 h	75–100	Gupta et al., 1993b
14 mg/24 h (Nicoderm)	11–16	4–7 h		Gupta et al., 1993a
21 mg/24 h (Nicoderm)	18–23	3–7 h	68	Prather et al., 1993
21 mg/24 h (Habitrol)	12–21	9–12 h	82	Gupta et al., 1995; Benowitz et al., 1997; Fant et al., 2000
Subcutaneous injection 2.4 mg	15	25	100	Le Houezec et al., 1993
Oral capsule 3–4 mg	6–8	90	44	Benowitz et al., 1991b
Oral slow-release capsule (colonic absorption) 6 mg	2.2	7.5 h		Green et al., 1999
Oral solution				
2 mg	4.7	51		Dempsey et al., 2004
~3.0 mg (45 μg/kg)	2.9	66	20	Zins et al., 1997
Enema				Zins et al., 1997
~3.5 mg (45 μg/kg)	2.3–3.1	20–80	15–25	Green et al., 1997
6 mg	6–9	45		Green et al., 1998

[a] Products in bold are currently marketed in the United States.
[b] C_{max} and T_{max} values are for peripheral venous blood unless otherwise indicated.
[c] T_{max} values are measured from the start of the administration.
[d] Estimated dose of 2 mg of nicotine per cigarette is higher than the usual 1 to 1.5 mg per cigarette since nicotine absorption from smoking a single cigarette was studied after at least overnight abstinence from smoking in these studies.

pass metabolism when using other NRTs, inhaler, sublingual tablets, nasal spray, and lozenges (Johansson et al., 1991; Bergstrom et al., 1995; Lunell et al., 1996; Molander and Lunell, 2001; Choi et al., 2003). Bioavailability for these products with absorption mainly through the mucosa of the oral cavity and a considerable swallowed portion is about 50 to 80% (Table 1).

Nicotine is poorly absorbed from the stomach because it is protonated (ionized) in the acidic gastric fluid, but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area. Following the administration of nicotine capsules or nicotine in solution, peak concentrations are reached in about 1 h (Benowitz et al., 1991; Zins et al., 1997; Dempsey et al., 2004). The oral bioavailability of nicotine is about 20 to 45% (Benowitz et al., 1991; Compton et al., 1997; Zins et al., 1997). Oral bioavailability is incomplete because of the hepatic first-pass metabolism. Also the bioavailability after colonic (enema) administration of nicotine (examined as a potential therapy for ulcerative colitis) is low, around 15 to 25%, presumably due to hepatic first-pass metabolism (Zins et al., 1997). Cotinine is much more polar than nicotine, is metabolized more slowly, and undergoes little, if any, first-pass metabolism after oral dosing (Benowitz et al., 1983b; De Schepper et al., 1987; Zevin et al., 1997).

Nicotine base is well absorbed through the skin. That is the reason for the occupational risk of nicotine poisoning (green tobacco sickness) in tobacco harvesters who are exposed to wet tobacco leaves (McBride et al., 1998). That is also the basis for transdermal delivery technology (Benowitz, 1995). Currently in the United States several different nicotine transdermal systems are marketed. All are multilayer patches. The rate of release of nicotine into the skin is controlled by the permeability of the skin, rate of diffusion through a polymer matrix, and/or rate of passage through a membrane in the various patches. The rates of nicotine delivery and plasma nicotine concentrations vary among the different transdermal systems (Fig. 3) (Fant et al., 2000). In all cases, there is an initial lag time of about 1 h before nicotine appears in the bloodstream, and there is continued systemic absorption (about 10% of the total dose) after the patch is removed, the latter due to residual nicotine in the skin.

IV. Distribution of Nicotine in Body Tissues

After absorption, nicotine enters the bloodstream where, at pH 7.4, it is about 69% ionized and 31% unionized. Binding to plasma proteins is less than 5% (Benowitz et al., 1982a). The drug is distributed extensively to body tissues with steady-state volume of distribution averaging 2.6 body weight (Table 2). Based on human autopsy samples from smokers, the highest affinity for nicotine is in the liver, kidney, spleen, and lung and the lowest affinity in adipose tissue (Urakawa et al., 1994). Cotinine concentrations are highest in the liver. In skeletal muscle the concentrations of nicotine and cotinine are close to that of whole blood. In a large postmortem case series of suicides and homicides with nicotine solution sold as a pesticide, brain and kidney nicotine levels were 75 to 80% of the level in liver, whereas blood levels were as high as in the liver (Grusz-Harday, 1967). In one fatal case of intentional overdose of nicotine patches, brain nicotine levels were about 2-fold higher than in peripheral blood and about 40% of the nicotine level in the liver (Kemp et al., 1997). Nicotine binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Benwell et al., 1988; Breese et al., 1997; Court et al., 1998; Perry et al., 1999). The increase in the binding is caused by a higher number of nicotinic cholinergic receptors in the brain of the smokers. Nicotine accumu-

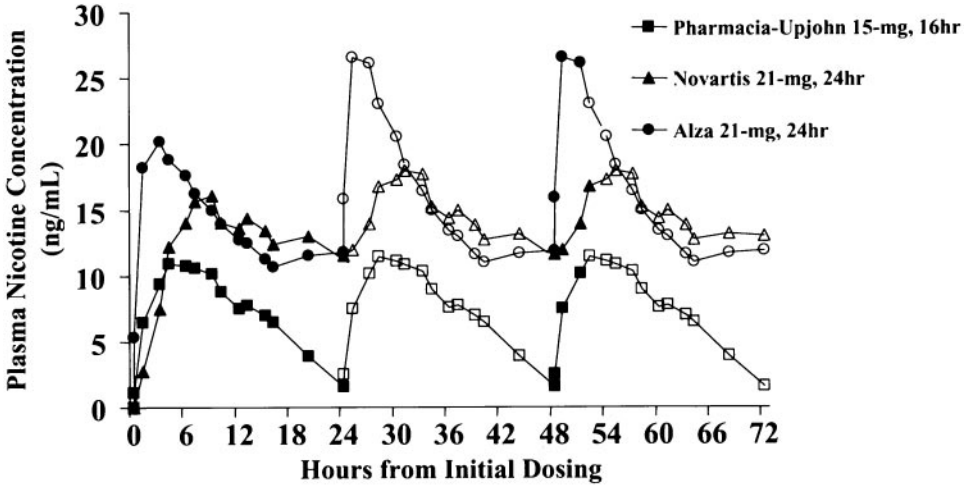

FIG. 3. Mean nicotine plasma concentrations during exposure to three different transdermal nicotine systems. Data points from 0 to 24 h are actual nicotine plasma concentrations (filled points). Data points from 24 to 72 h are nicotine plasma concentrations generated using the method of superposition (unfilled points). Pharmacia-Upjohn, Nicotrol; Novartis, Habitrol; Alza, Nicoderm (Reprinted from Fant et al., 2000 with permission from Elsevier).

TABLE 2
Pharmacokinetic parameters of (S)-nicotine, (S)-cotinine and (3′R,5′S)-trans-3′-hydroxycotinine after intravenous administration

	Clearance	Renal Clearance	Nonrenal Clearance	Volume of Distribution (Steady State)	$t_{1/2}$[a]	References
		ml/min		*l/kg*	*min*	
Nicotine	1110–1500	35–90	1050–1460	2.2–3.3	100–150	Benowitz and Jacob, 1993, 1994, 2000; Zevin et al., 1997; Benowitz et al., 1999, 2002a
Cotinine	42–55	3–9	36–52	0.69–0.93	770–1130	Benowitz and Jacob, 1994, 2000; Zevin et al., 1997; Benowitz et al., 1999, 2002a
Trans-3′-hydroxycotinine	82	50	32	0.66	396	Benowitz and Jacob, 2001

[a] $t_{1/2}$, elimination half-life.

lates markedly in gastric juice and saliva (Russell and Feyerabend, 1978; Lindell et al., 1993, 1996). Gastric juice/plasma and saliva/plasma concentration ratios are 61 and 11 with transdermal nicotine administration, and 53 and 87 with smoking, respectively (Lindell et al., 1993, 1996). The accumulation is caused by ion-trapping of nicotine in gastric juice and saliva. Nicotine also accumulates in breast milk (milk/plasma ratio 2.9) (Luck and Nau, 1984; Dahlstrom et al., 1990). Nicotine crosses the placental barrier easily, and there is evidence for the accumulation of nicotine in fetal serum and amnionic fluid in slightly higher concentrations than in maternal serum (Luck et al., 1985; Pastrakuljic et al., 1998; Dempsey and Benowitz, 2001).

The time course of nicotine in the brain and in other body organs and resultant pharmacologic effects are highly dependent on the route and rate of dosing. Smoking a cigarette delivers nicotine rapidly to the pulmonary venous circulation, from which it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and to the brain. The lag time between a puff of a cigarette and nicotine reaching the brain is 10 to 20 s. Although the delivery of nicotine to the brain is rapid, there is nevertheless significant pulmonary uptake and some delayed release of nicotine as evidenced by pulmonary positron emission tomography data and the slow decrease in the arterial concentrations of nicotine between puffs (Lunell et al., 1996; Rose et al., 1999). Nicotine concentrations in arterial blood after smoking a cigarette can be quite high, reaching up to 100 ng/ml, but usually ranging between 20 and 60 ng/ml (Armitage et al., 1975; Henningfield et al., 1993; Gourlay and Benowitz, 1997; Rose et al., 1999; Lunell et al., 2000). The usual peak arterial nicotine concentration after the first puff is lower, averaging 7 ng/ml (Rose et al., 1999). As high as 10-fold arterial/venous nicotine concentration ratios have been measured (Henningfield et al., 1993), but the mean ratio is typically around 2.3 to 2.8 (Gourlay and Benowitz, 1997; Rose et al., 1999). The rapid rate of delivery of nicotine by smoking (or intravenous injection, which presents similar distribution kinetics) results in high levels of nicotine in the central nervous system with little time for development of tolerance. The result is a more intense pharmacologic action (Porchet et al., 1987). The short time interval between puffing and nicotine entering the brain also allows the smoker to titrate the dose of nicotine to a desired pharmacologic effect, further reinforcing drug self-administration and facilitating the development of addiction.

In contrast, slow delivery of nicotine, such as by transdermal systems, results in little, if any, arterial-venous disequilibrium. The resultant brain levels of nicotine are much lower than after smoking, and the gradual rise in levels of nicotine in the central nervous system allows for the development of considerable tolerance to pharmacologic effects. Thus, the intensity of central nervous system effects is much less, and the addiction liability with the use of transdermal nicotine is virtually nil (Henningfield and Keenan, 1993). Routes of dosing that are associated with more rapid rates of delivery, such as nasal spray, are expected to result in higher intensity of effects and higher addiction liability when compared with products with slower absorption. Some indications of this were seen in a recent study comparing the abuse liability of the nicotine patch, gum, nasal spray, and inhaler in smoking cessation (West et al., 2000). Nasal spray had the highest rate of continuing use at the end of the study compared with the other NRTs; however, overall abuse liability was low for all products. These same considerations regarding rate of delivery and pharmacologic effects are expected to apply to nicotine-related compounds.

V. Nicotine and Cotinine Blood Levels during Tobacco Use and Nicotine Replacement Therapy

Blood or plasma nicotine concentrations sampled in the afternoon in smokers generally range from 10 to 50 ng/ml (Benowitz et al., 1990). Typical trough concentrations during daily smoking are 10 to 37 ng/ml, and typical peak concentrations range between 19 and 50 ng/ml (Schneider et al., 2001). The increment in venous blood nicotine concentration after smoking a single cigarette ranges from 5 to 30 ng/ml, depending on how a cigarette is smoked. In a recent study, the mean nicotine boost after smoking a cigarette was 10.9 ng/ml in smok-

ers with no smoking abstinence on the study day (Patterson et al., 2003). Blood levels peak at the end of smoking a cigarette and decline rapidly over the next 20 min due to tissue distribution. The distribution half-life averages about 8 min.

Peak venous blood levels of nicotine are similar, although the rate of rise of nicotine is slower, for cigar smokers and users of snuff and chewing tobacco compared with cigarette smokers (Armitage et al., 1978; Benowitz et al., 1988). Pipe smokers, particularly those who have previously smoked cigarettes, may have blood and urine levels of nicotine as high as cigarette smokers (Turner et al., 1977; Wald et al., 1981; McCusker et al., 1982). Primary pipe smokers who have not previously smoked cigarettes tend to have lower nicotine levels. Likewise, cigar smokers who have previously smoked cigarettes may inhale more deeply and achieve higher blood levels of nicotine than primary cigar smokers (Armitage et al., 1978), although on average, based on urinary cotinine levels, daily nicotine intake appears to be less for cigar compared with cigarette or pipe smokers (Wald et al., 1984).

The plasma half-life of nicotine after intravenous infusion or cigarette smoking averages about 2 h (Table 2). However, when half-life is determined using the time course of urinary excretion of nicotine, which is more sensitive in detecting lower levels of nicotine in the body, the terminal half-life averages 11 h (Jacob et al., 1999). The longer half-life detected at lower concentrations of nicotine is most likely a consequence of slow release of nicotine from body tissues. Based on a half-life of 2 h for nicotine, one would predict accumulation over 6 to 8 h (3 to 4 half-lives) of regular smoking and persistence of significant levels for 6 to 8 h after cessation of smoking. If a smoker smokes until bedtime, significant levels should persist all night. Studies of blood levels in regular cigarette smokers confirm these predictions (Fig. 4) (Benowitz et al., 1982b). Peak and trough levels follow each cigarette, but as the day progresses, trough levels rise and the influence of peak levels become less important. Thus, nicotine is not a drug to which smokers are exposed intermittently and which is eliminated rapidly from the body. To the contrary, smoking represents a multiple dosing situation with considerable accumulation while smoking and persistent levels for 24 h of each day.

Plasma levels of nicotine from nicotine replacement therapies tend to be in the range of low-level cigarette smokers. Thus, typical steady-state plasma nicotine concentrations with nicotine patches range from 10 to 20 ng/ml, for nicotine gum, inhaler, sublingual tablet, and nasal spray from 5 to 15 ng/ml (Benowitz et al., 1987, 1995; Schneider et al., 2001). Usually ad libitum use of NRTs results in one-third to two-thirds the concentration of nicotine that is achieved by cigarette smoking (Benowitz, 1993; Schneider et al., 2001). However, users of 4-mg nicotine gum may sometimes reach or even

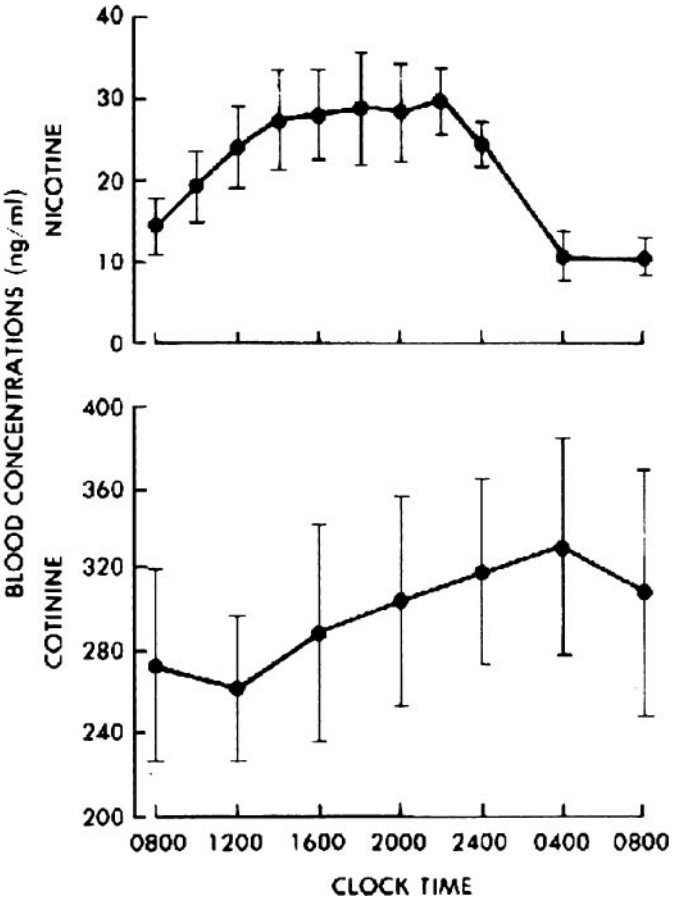

FIG. 4. Circadian blood concentrations of nicotine and cotinine during unrestricted smoking. Data are mean ± S.E.M. for eight subjects. Reprinted from Benowitz et al., 1983b with permission from American Society for Clinical Pharmacology and Therapeutics.

exceed the nicotine levels associated with smoking (McNabb et al., 1982, 1984). For the sake of comparison, systemic doses from various nicotine delivery systems are as follows: cigarette smoking, 1 to 2 mg per cigarette (Benowitz and Jacob, 1984; Jarvis et al., 2001); nicotine gum, 2 mg for a 4-mg gum (Benowitz et al., 1988; Stevens, 1994); transdermal nicotine, 5 to 21 mg per day, depending on the patch (Table 1); nicotine nasal spray, 0.7 mg per 1-mg dose of one spray in each nostril (Johansson et al., 1991; Gourlay and Benowitz, 1997); nicotine inhaler, 2 mg for a 4-mg dose released from the 10-mg inhaler (Molander et al., 1996); nicotine lozenge, 1 mg for a 2-mg lozenge (Choi et al., 2003); oral snuff, 3.6 mg for 2.5 g held in the mouth for 30 min (Benowitz et al., 1988); and chewing tobacco, 4.5 mg for 7.9 g chewed for 30 min (Benowitz et al., 1988).

Cotinine is present in the blood of smokers in much higher concentrations than those of nicotine. Cotinine blood concentrations average about 250 to 300 ng/ml in groups of cigarette smokers (Benowitz et al., 1983a; Gori and Lynch, 1985). We have seen levels in tobacco users ranging up to 900 ng/ml. After stopping smoking, levels decline in a log linear fashion with an average half-life of about 16 h (Table 2). The half-life of cotinine derived

from nicotine is longer than the half-life of cotinine administered as cotinine (Zevin et al., 1997). This is caused by slow release of nicotine from tissues. Because of the long half-life there is much less fluctuation in cotinine concentrations throughout the day compared with nicotine concentrations. As expected, there is a gradual rise in cotinine levels throughout the day, peaking at the end of smoking and persisting at high concentrations overnight (Fig. 4). Because of the long half-life of cotinine, it has been used as a biomarker for daily intake, both in cigarette smokers and in those exposed to environmental tobacco smoke (Benowitz, 1996a). There is a high correlation among cotinine concentrations measured in plasma, saliva, and urine, and measurements in any one of these fluids can be used as a marker of nicotine intake. Cotinine levels produced by NRTs are usually 30 to 70% of the levels detected while smoking (Hurt et al., 1994; Schneider et al., 1995, 1996).

VI. Metabolism of Nicotine

A. Primary Metabolites of Nicotine

Studies of nicotine metabolism have been conducted over several decades, and different authors have used different names for the same metabolite. The most frequently used alternative names for nicotine and many of its metabolites are listed in Table 3. Since Chemical Abstracts provides access to much of the world's literature on chemistry and pharmacology, Chemical Abstracts Registry numbers and index names are listed in this table.

Nicotine is extensively metabolized to a number of metabolites (Fig. 5) by the liver. Six primary metabolites of nicotine have been identified. Quantitatively, the most important metabolite of nicotine in most mammalian species is the lactam derivative cotinine. In humans, about 70 to 80% of nicotine is converted to cotinine (Benowitz and Jacob, 1994). This transformation involves two steps. The first is mediated by a cytochrome P450 system to produce nicotine-$\Delta^{1'(5')}$-iminium ion, which is in equilibrium with 5′-hydroxynicotine (Murphy, 1973; Brandange and Lindblom, 1979b; Peterson et al., 1987). The second step is catalyzed by a cytoplasmic aldehyde oxidase (Brandange and Lindblom, 1979a; Gorrod and Hibberd, 1982). Nicotine iminium ion has received considerable interest since it is an alkylating agent and, as such, could play a role in the pharmacology of nicotine (Gorrod and Jenner, 1975; Hibberd and Gorrod, 1981; Shigenaga et al., 1988; Jacob et al., 1997).

Nicotine *N*′-oxide is another primary metabolite (Fig. 6) of nicotine, although only about 4 to 7% of nicotine absorbed by smokers is metabolized via this route (Byrd et al., 1992; Benowitz et al., 1994). The conversion of nicotine to nicotine *N*′-oxide involves a flavin-containing monooxygenase 3 (FMO3), which results in formation of both possible diasteriomers, the 1′-(*R*)-2′-(*S*)-*cis* and 1′-(*S*)-2′-(*S*)-*trans*-isomers in animals (Cashman et al., 1992; Park et al., 1993). In humans, this pathway is highly selective for the *trans*-isomer (Cashman et al., 1992). Only the *trans*-isomer of nicotine *N*′-oxide was detected in urine after administration of nicotine by intravenous infusion, transdermal patch, or smoking (Park et al., 1993). It appears that nicotine *N*′-oxide is not further metabolized to any significant extent, except by reduction back to nicotine, which may lead to recycling of nicotine in the body (Dajani et al., 1975). A study by Beckett et al. (1970) indicated that reduction of nicotine *N*′-oxide to nicotine in humans is mediated by bacterial action in the large intestine. These investigators found that nicotine *N*′-oxide administered intravenously was excreted largely, if not entirely, in the urine unchanged, whereas administration rectally as an enema resulted in extensive conversion to nicotine and cotinine, which appeared in the urine. Oral administration of nicotine *N*′-oxide resulted in small but significant urinary excretion of nicotine and cotinine.

In addition to oxidation of the pyrrolidine ring, nicotine is metabolized by two nonoxidative pathways, methylation of the pyridine nitrogen giving nicotine isomethonium ion (also called *N*-methylnicotinium ion) and glucuronidation (Fig. 6). The methylation pathway was first reported by McKennis, who found it in dogs dosed with (*S*)-nicotine (McKennis et al., 1963a). Studies of the *N*-methylation pathway using animal models and human liver homogenates show that *S*-adenosyl-L-methionine is the source of the methyl group in a reaction catalyzed by the amine *N*-methyltransferase (Crooks and Godin, 1988; Nwosu and Crooks, 1988). Human liver cytosol was capable of methylating both enantiomers of nicotine, but the (*R*)-isomer was methylated more rapidly than the (*S*)-isomer. Small amounts of nicotine isomethonium ion have been detected in smokers' urine (Neurath et al., 1987, 1988). In light of the reported pharmacologic activity of nicotine isomethonium ion (Dwoskin et al., 1992), further studies of this metabolite are warranted.

Nicotine glucuronidation results in an *N*-quaternary glucuronide in humans (Curvall et al., 1991; Byrd et al., 1992; Benowitz et al., 1994). This reaction is catalyzed by uridine diphosphate-glucuronosyltransferase (UGT) enzyme(s) producing (*S*)-nicotine-*N*-β-glucuronide (Seaton et al., 1993). About 3 to 5% of nicotine is converted to nicotine glucuronide and excreted in urine in humans.

Oxidative *N*-demethylation is frequently an important pathway in the metabolism of xenobiotics, but this route is, in most species, a minor pathway in the metabolism of nicotine. Conversion of nicotine to nornicotine in humans has been demonstrated. We found that small amounts of deuterium-labeled nornicotine are excreted in urine of smokers administered deuterium-labeled nicotine (Jacob and Benowitz, 1991). Metabolic formation of nornicotine from nicotine has also been reported by Neurath et al. (1991). Nornicotine is a constituent of tobacco leaves. However, the majority of urine nornico-

TABLE 3
Some commonly used alternative names for nicotine and metabolites

	Alternative Names
Nicotine (54-11-5)[a]	(*S*)-Nicotine (−)-Nicotine l-Nicotine 3-(1-Methyl-2-pyrrolidinyl)pyridine CA[b]: Pyridine, 3-[(2*S*)-1-methyl-2-pyrrolidinyl]-
Cotinine (486-56-6)	(*S*)-Cotinine (−)-Cotinine 1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone CA: 2-Pyrrolidinone, 1-methyl-5-(3-pyridinyl)-, (5*S*)-
Trans-3′-hydroxycotinine (34834-67-8)	Hydroxycotinine 3′-Hydroxycotinine 3-Hydroxy-1-methyl-5-(3-pyridinyl)-2-pyrrolidinone CA: 2-Pyrrolidinone, 3-hydroxy-1-methyl-5-(3-pyridinyl)-, (3*R*,5*S*)-
Nicotine *N*′-oxide (491-26-9; *trans*: 51095-86-4)	Nicotine 1′-oxide Nicotine 1′-*N*-oxide 1′(*S*)-2′(*S*)-*Trans*-nicotine-*N*′-oxide CA: Pyridine, 3-[(2*S*)-1-methyl-1-oxido-2-pyrrolidinyl]- *Trans*, CA: Pyridine, 3-[(1*S*,2*S*)-1-methyl-1-oxido-2-pyrrolidinyl]-
Nornicotine (*S*-Isomer: 494-97-3; Racemic: 5746-86-1)	*S*-Isomer: (−)-Nornicotine l-Nornicotine *S*-Isomer, CA: Pyridine, 3-(2*S*)-2-pyrrolidinyl- Racemic: (±)-Nornicotine (*RS*)-Nornicotine Racemic, CA: Pyridine, 3-(2-pyrrolidinyl)-
Norcotinine (*S*-Isomer: 5980-06-3; Racemic: 17708-87-1)	Demethylcotinine *S*-Isomer: (−)-Demethylcotinine *S*-(−)-Norcotinine *S*-Isomer, CA: 2-Pyrrolidinone, 5-(3-pyridinyl)-, (5*S*)- Racemic: (±)-Demethylcotinine (±)-Norcotinine (*RS*)-Norcotinine Racemic, CA: 2-Pyrrolidinone, 5-(3-pyridinyl)-
Cotinine *N*-oxide (36508-80-2)	CA: 2-Pyrrolidinone, 1-methyl-5-(1-oxido-3-pyridinyl)-, (5*S*)-
5′-Hydroxycotinine (Racemic: 75919-05-0; 5′(*R*): 61192-50-5)	Allohydroxycotinine Racemic, CA: 2-Pyrrolidinone, 5-hydroxy-1-methyl-5-(3-pyridinyl)- 5′(*R*), CA: 2-Pyrrolidinone, 5-hydroxy-1-methyl-5-(3-pyridinyl)-, (5*R*)-
4-Oxo-4-(3-pyridyl)-*N*-methylbutanamide (713-05-3)	γ-(3-Pyridyl)-γ-oxo-*N*-methylbutyramide CA: 3-Pyridinebutanamide, *N*-methyl-γ-oxo-
5′-Hydroxynorcotinine (118995-82-7)	Allohydroxynorcotinine CA: 2-Pyrrolidinone, 5-hydroxy-5-(3-pyridinyl)-
4-Oxo-4-(3-pyridyl)-butanamide (61192-49-2)	4-(3-Pyridyl)-4-oxobutyramide CA: 3-Pyridinebutanamide, γ-oxo-
4-Oxo-4-(3-pyridyl)butanoic acid (4192-31-8)	γ-(3-Pyridyl)-γ-oxobutyric acid 4-(3-Pyridyl)-4-oxobutyric acid CA: 3-Pyridinebutanoic acid, γ-oxo-
4-Hydroxy-4-(3-pyridyl)butanoic acid (15569-97-8)	γ-(3-Pyridyl)-γ-hydroxybutyric acid 4-(3-Pyridyl)-4-hydroxybutyric acid CA: 3-Pyridinebutanoic acid, γ-hydroxy-
4-(Methylamino)-1-(3-pyridyl)-1-butanone, and cyclized form (2055-23-4); Cyclized and dehydrated forms (525-74-6, 75043-32-2)	Pseudooxynicotine Cyclized form: 2′-hydroxynicotine Cyclized and dehydrated: *N*-methylmyosmine CA: 1-Butanone, 4-(methylamino)-1-(3-pyridinyl)- Cyclized and dehydrated forms, CA: Pyridine, 3-(4,5-dihydro-1-methyl-1*H*-pyrrol-2-yl)- and 2*H*-Pyrrolium, 3,4-dihydro-1-methyl-5-(3-pyridinyl)-
Nicotine isomethonium ion (21446-46-8)	*N*-methylnicotinium ion CA: Pyridinium, 1-methyl-3-[(2*S*)-1-methyl-2-pyrrolidinyl]-, iodide
Cotinine methonium ion (33952-07-7)	*N*-methylcotininium ion CA: Pyridinium, 1-methyl-3-(1-methyl-5-oxo-2-pyrrolidinyl)-, iodide, (*S*)-

[a] The numbers in parentheses are Chemical Abstracts Registry numbers.
[b] CA, Chemical Abstracts index name.

tine is derived from the metabolism of nicotine with less than 40% coming directly from tobacco, as estimated from the difference in nornicotine excretion in smokers during smoking and during transdermal nicotine treatment (0.65 and 0.41%, respectively) (Benowitz et al., 1994). The formation of nornicotine from nicotine has been shown to be mediated by cytochrome P450 system in rabbits (Williams et al., 1990b). Formation of an iminium ion as an intermediate in the demethylation of nicotine was reported by Castagnoli and coworkers, who characterized *N*′-cyanomethylnornicotine in extracts obtained following incubation of rabbit liver microsomes with nicotine and sodium cyanide (Nguyen et al., 1979). This observation implies the intermediacy of *N*′-methylene-iminium ion, which is captured by cyanide ion to form the stable cyano adduct. In the absence of cyanide, the iminium ion would be expected to hydrolyze to nornicotine and formaldehyde.

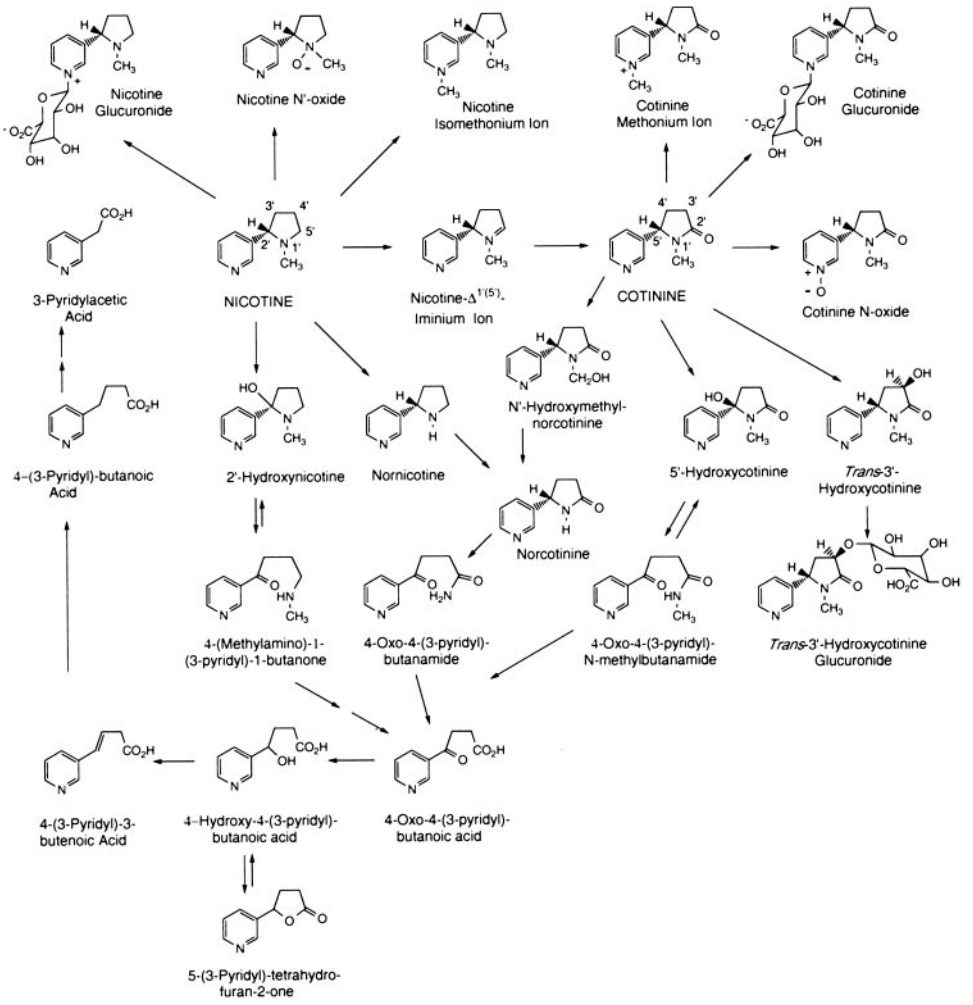

FIG. 5. Pathways of nicotine metabolism.

A new cytochrome P450 mediated metabolic pathway for nicotine metabolism was recently reported by Hecht et al. (2000). 2′-Hydroxylation of nicotine was shown to produce 4-(methylamino)-1-(3-pyridyl)-1-butanone with 2′-hydroxynicotine as an intermediate (Fig. 5). 2′-Hydroxynicotine also yields nicotine-$\Delta^{1'(2')}$-iminium ion. 4-(Methylamino)-1-(3-pyridyl)-1-butanone is further metabolized to 4-oxo-4-(3-pyridyl)butanoic acid and 4-hydroxy-4-(3-pyridyl)butanoic acid. Previously these metabolites were thought to arise mainly through metabolism of cotinine (McKennis et al., 1964). The new pathway is potentially significant since 4-(methylamino)-1-(3-pyridyl)-1-butanone can be converted to carcinogenic NNK. However, endogenous production of NNK from nicotine has not been detected in humans or rats (Carmella et al., 1997; Hecht et al., 1999a).

About 10 to 15% of nicotine and metabolites is excreted as 4-oxo-4-(3-pyridyl)butanoic acid and 4-hydroxy-4-(3-pyridyl)butanoic acid in smokers' urine (Hecht et al., 1999b,c). Less than 0.5% of cotinine dosed to nonsmokers was recovered as these metabolites, demonstrating that the 2′-hydroxylation of nicotine is the primary, although not the only, pathway for their production (unpublished data reported in Hecht et al., 2000). 4-Hydroxy-4-(3-pyridyl)butanoic acid is further metabolized to 3-pyridylacetic acid, the so-called terminal metabolite of nicotine. It has been detected in human urine following oral administration of cotinine (McKennis et al., 1964). It is speculated (McKennis et al., 1964) that 3-pyridylacetic acid is formed via dehydration of the hydroxyacid 4-hydroxy-4-(3-pyridyl)butanoic acid to give 4-(3-pyridyl)-3-butenoic acid, reduction to 4-(3-pyridyl)-butanoic acid, β-oxidation, and cleavage to 3-pyridylacetic acid. This metabolic scheme is analogous to the catabolism of fatty acids, although there is no experimental evidence for the intermediacy of 4-(3-pyridyl)-3-butenoic acid or of 4-(3-pyridyl)-butanoic acid.

B. Cotinine Metabolism

Although on average about 70 to 80% of nicotine is metabolized via the cotinine pathway in humans, only 10 to 15% of the nicotine absorbed by smokers appears in the urine as unchanged cotinine (Benowitz et al., 1994). A number of cotinine metabolites have been structurally characterized (Fig. 5). Indeed, it appears that most of the reported urinary metabolites of nicotine are derived

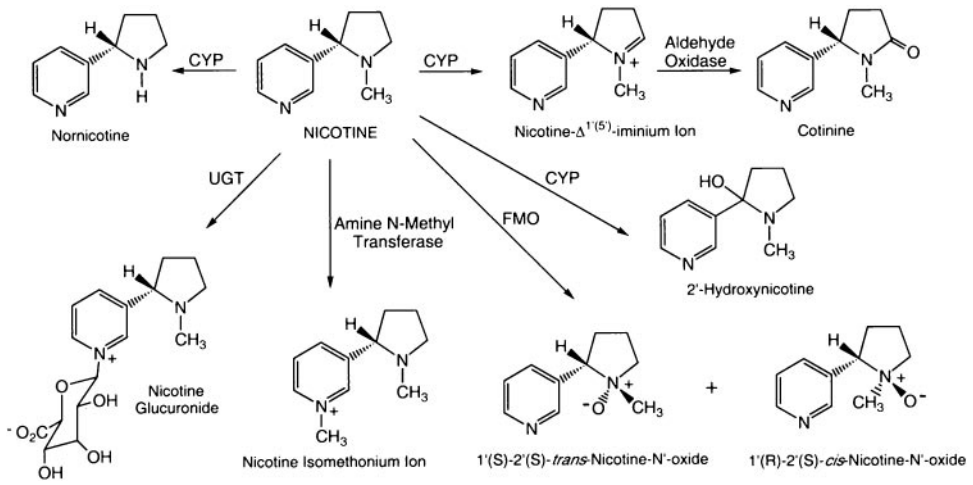

FIG. 6. Primary routes of nicotine metabolism.

from cotinine. Six primary metabolites of cotinine have been reported in humans: 3′-hydroxycotinine (Bowman and McKennis, 1962; McKennis et al., 1963b; Neurath et al., 1987), 5′-hydroxycotinine (also called allohydroxycotinine) (Neurath, 1994), which exists in tautomeric equilibrium with the open chain derivative 4-oxo-4-(3-pyridyl)-*N*-methylbutanamide (Bowman and McKennis, 1962; McKennis et al., 1962), cotinine *N*-oxide (Shulgin et al., 1987; Kyerematen et al., 1990b), cotinine methonium ion (McKennis et al., 1963a), cotinine glucuronide (Curvall et al., 1991; Caldwell et al., 1992), and norcotinine (also called demethylcotinine) (Bowman et al., 1959; Kyerematen et al., 1990b).

3′-Hydroxycotinine is the main nicotine metabolite detected in smokers' urine. It is also excreted as a glucuronide conjugate (Fig. 5) (Curvall et al., 1991; Benowitz et al., 1994). 3′-Hydroxycotinine and its glucuronide conjugate account for 40 to 60% of the nicotine dose in urine (Byrd et al., 1992; Benowitz et al., 1994; Andersson et al., 1997; Hecht et al., 1999b). The conversion of cotinine to 3′-hydroxycotinine in humans is highly stereoselective for the *trans*-isomer, as less than 5% is detected as *cis*-3′-hydroxycotinine in urine (Jacob et al., 1990; Voncken et al., 1990). Although nicotine and cotinine conjugates are *N*-glucuronides, the only 3′-hydroxycotinine conjugate detected in urine is the *O*-glucuronide (Byrd et al., 1994). Recently, significant rates of *N*-glucuronidation of 3′-hydroxycotinine were detected in human liver microsomes, but this metabolite was not detected in urine (Kuehl and Murphy, 2003b). Thus, the *N*-glucuronide of 3′-hydroxycotinine might be unstable, or the concentration was too low to detect the *N*-glucuronide by the methodology employed.

As with nicotine *N*′-oxide, cotinine *N*-oxide can be reduced back to the parent amine in vivo as evidenced by a study in rabbits (Yi et al., 1977). Studies with P450 enzyme inhibitors in hamster and guinea pig liver microsomes show that, unlike nicotine *N*′-oxide, cotinine *N*-oxide is formed by P450 enzymes (Hibberd and Gorrod, 1985). Cotinine *N*-oxide accounts for 2 to 5% of the nicotine and metabolites in smokers' urine (Byrd et al., 1992; Benowitz et al., 1994; Meger et al., 2002). McKennis and coworkers showed that cotinine methonium ion is excreted in urine after cotinine administration to a single subject (McKennis et al., 1963a).

Norcotinine has been detected in smokers' urine (about 1% of total nicotine and metabolites) (Byrd et al., 1992, 1995b). Two pathways for its formation are possible, demethylation of cotinine or oxidative metabolism of nornicotine (Fig. 5). Animal studies have demonstrated the existence of both of these pathways. Wada et al. (1961) and Papadopoulos (1964) reported that norcotinine is a metabolite of nornicotine, and Harke et al. (1974) detected norcotinine in urine of pigs following cotinine administration. From studies in which deuterium-labeled nicotine and cotinine were administered, we have obtained evidence that both pathways occur in humans as well (P. Jacob III, L. Yu, and N. L. Benowitz, unpublished data). *N*′-Hydroxymethylnorcotinine has been detected in vitro in hamster hepatic microsomes after incubation with cotinine in some (Li and Gorrod, 1994) but not all (Murphy et al., 1999) studies. It is speculated to be an intermediate in the conversion of cotinine to norcotinine (Li and Gorrod, 1994). In rat liver microsomes, norcotinine is further metabolized to 4-oxo-4-(3-pyridyl)-butanamide (Eldirdiri et al., 1997). 4-Oxo-4-(3-pyridyl)-butanamide is metabolized further to 4-oxo-4-(3-pyridyl)butanoic acid. 4-Oxo-4-(3-pyridyl)-butanamide is in equilibrium with 5′-hydroxynorcotinine (allohydroxydemethylcotinine), dehydration of which leads to the formation of norcotinine $\Delta^{2'(3')}$-enamine (Kyerematen et al., 1990b). Using liquid chromatography-tandem mass spectrometry, we have found that small amounts of 4-oxo-4-(3-pyridyl)-butanamide (5′-hydroxynorcotinine) are present in smokers' urine (P. Jacob III, L. Yu, and N. L. Benowitz, unpublished data). Recently, the human CYP2A6 enzyme was shown to catalyze formation of norcotinine and 5′-hydroxycotinine from cotinine (Murphy et al., 1999).

5′-Hydroxycotinine has been detected in smokers' urine in levels of less than 4% of those of the 3′-hydroxycotinine (Neurath, 1994). 5′-Hydroxycotinine *N*-oxide has been isolated from rat urine after nicotine administration, but was unmeasurable in urine samples from smokers (Schepers et al., 1999). The ketoamide 4-oxo-4-(3-pyridyl)-*N*-methylbutanamide derived from 5′-hydroxycotinine is presumably the precursor of a number of nicotine metabolites which result from degradation of the pyrrolidine ring. These include the keto acid 4-oxo-4-(3-pyridyl)butanoic acid, its reduction product 4-hydroxy-4-(3-pyridyl)butanoic acid, a hydroxyacid which can be in equilibrium with the lactone 5-(3-pyridyl)-tetrahydrofuran-2-one, and 3-pyridylacetic acid, the so-called terminal metabolite of nicotine. As mentioned earlier, these metabolites are probably formed mainly via nicotine 2′-hydroxylation (Hecht et al., 2000).

Cotinine is a lactam, and it is reasonable to expect that the open-chain form γ-(3-pyridyl)-γ-methylaminobutyric acid [4-methylamino-4-(3-pyridyl)butanoic acid], an amino acid, might be an intermediate in cotinine formation or be formed via hydrolysis of cotinine. This open-chain derivative has indeed been reported to be present in urine of smokers (Bowman et al., 1959), but based on in vitro studies with liver homogenates, it appears that cotinine is formed directly by oxidation of the nicotine-$\Delta^{1'(5')}$-iminium ion (Brandange and Lindblom, 1979a; Gorrod and Hibberd, 1982) rather than via the amino acid.

C. Quantitative Aspects of Nicotine Metabolism

Quantitative aspects of the pattern of nicotine metabolism have been elucidated fairly well in people (Fig. 7). About 90% of a systemic dose of nicotine can be accounted for as nicotine and metabolites in the urine (Benowitz et al., 1994). Based on studies with simultaneous infusion of labeled nicotine and cotinine, it has been determined that 70 to 80% of nicotine is converted to cotinine (Benowitz and Jacob, 1994). About 4 to 7% of nicotine is excreted as nicotine *N*′-oxide and 3 to 5% as nicotine glucuronide (Byrd et al., 1992; Benowitz et al., 1994). Cotinine is excreted unchanged in the urine to a small degree (10 to 15% of the nicotine and metabolites in urine). The remainder is converted to metabolites, primarily *trans*-3′-hydroxycotinine (33–40%), cotinine glucuronide (12–17%), and *trans*-3′-hydroxycotinine glucuronide (7–9%).

The rate of metabolism of nicotine can be determined by measuring blood levels after administration of a known dose of nicotine. We have studied cigarette smokers and nonsmokers given intravenous infusions of nicotine for 30 to 60 min (Table 2). Total and renal clearance are computed directly and the nonrenal or metabolic clearance computed as the difference between the total and renal clearance. Total clearance of nicotine averages about 1200 ml/min. Nonrenal clearance represents about 70% of liver blood flow. Assuming most nicotine is metabolized by the liver, this means about

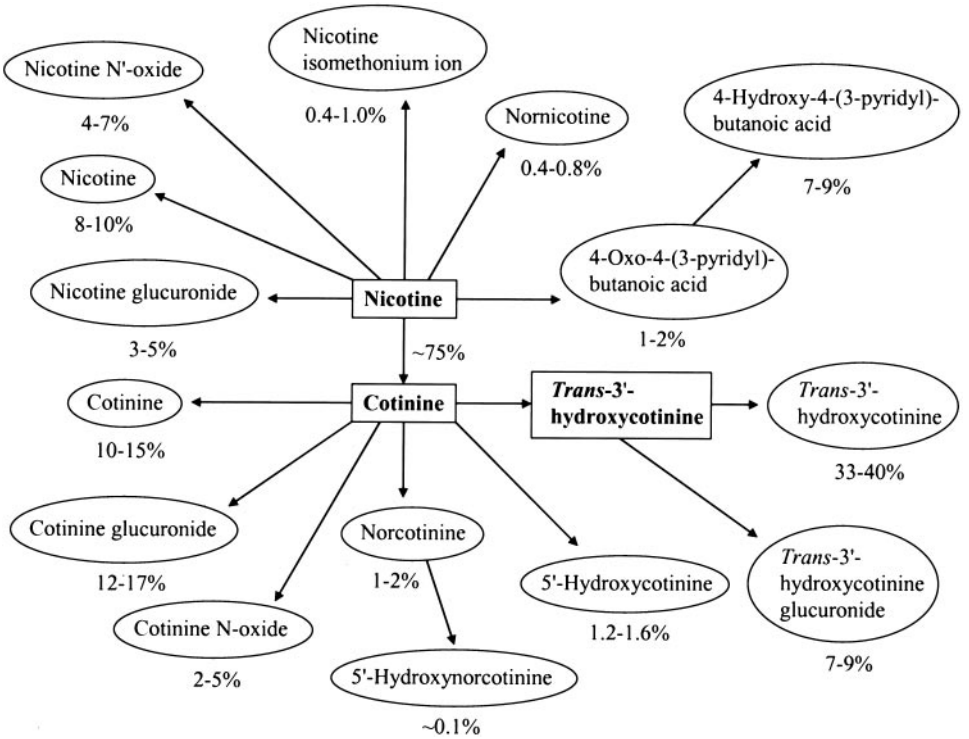

FIG. 7. Quantitative scheme of nicotine metabolism, based on estimates of average excretion of metabolites as percent of total urinary nicotine. As the data are gathered from several studies with differing methodologies, reader is advised to interpret them with caution. Data compiled from Neurath et al., 1987; Curvall et al., 1991; Byrd et al., 1992, 1995a,b; Benowitz et al., 1994; Neurath, 1994; Hecht et al., 1999b; Meger et al., 2002, and P. Jacob III, L. Yu, and N. L. Benowitz (unpublished data on 5′-hydroxynorcotinine).

70% of the drug is extracted from the blood in each pass through the liver.

The metabolism of cotinine is much slower than that of nicotine. Cotinine clearance averages about 45 ml/min (Table 2). The clearance of (3′*R*,5′*S*)-*trans*-3′-hydroxycotinine is also quite slow, about 82 ml/min. Because these compounds are metabolized slowly, the rates of elimination of cotinine and 3′-hydroxycotinine are predicted to not be substantially influenced by changes in liver blood flow.

Recently, we evaluated the measurement of the 3′-hydroxycotinine/cotinine ratio in plasma and saliva as a noninvasive probe for CYP2A6 activity (Dempsey et al., 2004). The ratio was highly correlated with the oral clearance of nicotine and the oral clearance and half-life of cotinine. Correlation coefficients of oral nicotine and cotinine clearances with plasma 3′-hydroxycotinine/cotinine ratios were 0.78 and 0.63, respectively, at 6 h after oral nicotine dosing. The 3′-hydroxycotinine/cotinine ratio could be used to phenotype nicotine metabolism and CYP2A6 enzyme in nonsmokers after oral nicotine dosing and in smokers after smoking regular cigarettes. We have previously shown that this ratio is a predictor of cigarette consumption (Benowitz et al., 2003).

D. Liver Enzymes Responsible for Nicotine and Cotinine Metabolism

1. Cytochrome P450 Enzymes. In vitro and in vivo studies show that CYP2A6 is the enzyme that is primarily responsible for the oxidation of nicotine and cotinine. The evidence for the role of CYP2A6 includes human liver microsome studies in which the rate and extent of nicotine oxidation to cotinine and cotinine oxidation to 3′-hydroxycotinine are highly correlated with coumarin 7-hydroxylase activity (known to be mediated by CYP2A6) and correlated with immunochemically determined hepatic CYP2A6 levels (Cashman et al., 1992; Berkman et al., 1995; Nakajima et al., 1996a,b). Nicotine oxidation to cotinine has also been shown to be inhibited by coincubation with coumarin (a competitive inhibitor of CYP2A6) and CYP2A6 antibodies (Nakajima et al., 1996b; Messina et al., 1997; Le Gal et al., 2003). CYP2A6 expression systems have high activity for metabolizing nicotine and cotinine (Nakajima et al., 1996a,b). Of note is that human liver specimens exhibit marked individual variability in levels of CYP2A6 mRNA and coumarin 7-hydroxylase activity, consistent with the known wide variability in the rate of nicotine metabolism in people (Pelkonen et al., 2000; Tyndale and Sellers, 2002). In addition to forming cotinine and 3′-hydroxycotinine, CYP2A6 is active in the 2′-hydroxylation of nicotine and in the formation of 5′-hydroxycotinine and norcotinine from cotinine (Murphy et al., 1999; Hecht et al., 2000).

In vivo studies support the significant role of CYP2A6 in nicotine metabolism. Methoxsalen, a CYP2A6 inhibitor, reduces the first-pass metabolism of oral nicotine, decreases clearance of subcutaneously administered nicotine, and decreases urinary levels of 3′-hydroxycotinine in smokers (Sellers et al., 2000, 2003a). Subjects homozygous for *CYP2A6* deletion allele (*CYP2A6*4*) have very low plasma cotinine levels after smoking or nicotine administration (Nakajima et al., 2000, 2001; Kwon et al., 2001; Xu et al., 2002b; Dempsey et al., 2004). Urinary excretion of cotinine in homozygotes for the deletion allele is only 10 to 30% of the levels detected in subjects with functional genes, depending on the experimental design of the study (Kitagawa et al., 1999; Yang et al., 2001; Zhang et al., 2002). No 3′-hydroxycotinine is detected in plasma and saliva after oral nicotine administration in subjects homozygous for *CYP2A6*4* (Dempsey et al., 2004). Also, urine 3′-hydroxycotinine excretion after smoking is markedly decreased in subjects with two *CYP2A6*4* alleles (Zhang et al., 2002). Thus, the metabolism of nicotine can be used to phenotype CYP2A6 activity (Nakajima et al., 2002a).

Although the studies mentioned above demonstrate the significant role of CYP2A6 in nicotine metabolism, they illustrate that other enzymes must also be involved in formation of cotinine and 3′-hydroxycotinine, at least in subjects lacking the CYP2A6 enzyme. CYP2B6 is the second most active hepatic P450 enzyme in nicotine *C*-oxidation when investigated using hepatic tissues or expression systems in vitro, especially at high nicotine concentrations (Flammang et al., 1992; McCracken et al., 1992; Nakajima et al., 1996b; Yamazaki et al., 1999). Most of the studies with CYP2D6 expression systems show some activity toward nicotine metabolism (McCracken et al., 1992; Nakajima et al., 1996b; Yamazaki et al., 1999; Le Gal et al., 2003), but there are also studies showing no cotinine formation by expressed CYP2D6 (Flammang et al., 1992; Messina et al., 1997). In humans, CYP2D6 poor metabolizer and extensive metabolizer phenotypes have similar nicotine and cotinine pharmacokinetics (Benowitz et al., 1996), although ultrarapid metabolizer phenotype caused by amplification of *CYP2D6* gene may be associated with accelerated nicotine metabolism (Saarikoski et al., 2000; Caporaso et al., 2001). CYP2E1 has some activity toward nicotine in in vitro systems at high nicotine concentrations (Flammang et al., 1992; Yamazaki et al., 1999; Le Gal et al., 2003). A preliminary report shows that heterologously expressed CYP2A13, a close relative of CYP2A6, has considerable activity toward nicotine and cotinine (Bao et al., 2000). CYP2A6 and CYP2A13 share several substrates, such as NNK and gasoline additive MTBE (methyl tertiary butyl ether) (Su et al., 2000). CYP2A13 is highly expressed in the human respiratory tract, especially in nasal mucosa (Getchell et al., 1993; Koskela et al., 1999; Su et al., 2000; Chen et al., 2003b). However, there is negligible expression of CYP2A13 in liver (Koskela et al., 1999; Su et al., 2000), indicating that it is probably of significance in nicotine clearance (if at all) only in subjects lacking functional CYP2A6 enzyme.

2. Aldehyde Oxidase. Aldehyde oxidase is a cytosolic enzyme catalyzing the conversion of nicotine-$\Delta^{1'(5')}$-imi-

nium ion to cotinine (Brandange and Lindblom, 1979a). In in vitro studies with rabbit liver microsomes, nicotine-$\Delta^{1'(5')}$-iminium ion accumulates in the absence of aldehyde oxidase (Obach and van Vunakis, 1990). Raloxifene, a selective estrogen receptor modulator and potent aldehyde oxidase inhibitor, inhibits the formation of cotinine in human liver cytosol with a K_i of 1.4 nM (Obach, 2004). In rat liver cytosol, aldehyde oxidase has been shown to catalyze the conversion of nicotine *N*′-oxide back to nicotine (Sugihara et al., 1996). There is no evidence for such a conversion in humans as nicotine *N*′-oxide administered intravenously is excreted entirely in the urine unchanged (Beckett et al., 1970).

3. Flavin-Containing Monooxygenase 3. FMO3 is the main enzyme responsible for nicotine *N*′-oxide formation (Fig. 6). FMO content of human liver microsomes is correlated with nicotine *N*′-oxide formation, and *N*′-oxygenase activity is completely abolished by FMO inhibitors (Cashman et al., 1992). Also, cDNA-expressed FMO3 is active in nicotine *N*′-oxide formation (Park et al., 1993). This pathway is highly selective for the production of *trans*-isomer. Thus, formation of (*S*)-nicotine *N*′-oxide can be used as a highly stereoselective probe of human FMO3 function (Cashman and Zhang, 2002). In vitro expressed P450 enzymes are not active in nicotine *N*′-oxide formation (Flammang et al., 1992). Dysfunctional FMO3 forms with deficient *N*-oxygenation of dietary trimethylamine cause trimethylaminuria (fish-odor syndrome) (Cashman and Zhang, 2002). Two preliminary reports have been published on the effect of impaired FMO3-mediated metabolism on nicotine *N*′-oxide formation. Two siblings with fish-odor syndrome were shown to have impaired urinary excretion of nicotine *N*′-oxide when compared with normal volunteers after nicotine chewing gum administration (Ayesh et al., 1988). Also, methimazole, an antithyroid agent and FMO3 inhibitor, was reported to reduce nicotine *N*′-oxide excretion after nicotine administration as nicotine chewing gum (Cholerton et al., 1988).

4. Amine N-Methyltransferase. Nicotine *N*-methylation is catalyzed by amine *N*-methyltransferase (Crooks and Godin, 1988; Nwosu and Crooks, 1988). The expression of this cytosolic enzyme is highest in human thyroid, adrenal gland, and lung (Thompson et al., 1999). It has some activity toward tryptamine *N*-methylation, but otherwise its metabolic functions are not characterized.

5. UDP-Glucuronosyltransferases. As described previously, nicotine and cotinine undergo phase II metabolic reactions via *N*-glucuronidation and 3′-hydroxycotinine mostly via *O*-glucuronidation. We have observed a high degree of correlation between the extent of glucuronide conjugation of nicotine and cotinine within individuals in vivo and no correlation between the extent of nicotine or cotinine versus 3′-hydroxycotinine conjugation (Benowitz et al., 1994). The correlation between nicotine and cotinine glucuronidation, and lack of correlation with 3′-hydroxycotinine conjugation, have also been detected in liver microsomes (Ghosheh and Hawes, 2002a,b; Nakajima et al., 2002b; Kuehl and Murphy, 2003a,b). Recently, significant rates of *N*-glucuronidation of 3′-hydroxycotinine were detected in human liver microsomes, but this metabolite was not detected in urine (Kuehl and Murphy, 2003b). In microsomes, *N*-glucuronidation of 3′-hydroxycotinine was correlated with *N*-glucuronidation of nicotine and cotinine. These results suggest that the same enzyme(s) that is responsible for the *N*-glucuronidation of nicotine and cotinine may also catalyze the *N*-glucuronidation of 3′-hydroxycotinine, but that a separate enzyme catalyzes 3′-hydroxycotinine *O*-glucuronidation.

Both nicotine and cotinine glucuronidation in human liver microsomes was inhibited by imipramine (UGT1A4 substrate), propofol (UGT1A9 substrate), and bilirubin (UGT1A1 substrate) (Nakajima et al., 2002b; Kuehl and Murphy, 2003a). Only imipramine glucuronidation was correlated with nicotine and cotinine conjugations in microsomes (Nakajima et al., 2002b). Heterologously expressed UGT1A4 and UGT1A9 are active in nicotine glucuronidation, whereas only UGT1A4 is active in cotinine glucuronidation (Kuehl and Murphy, 2003a). However, other studies with expressed UGT1A4 and UGT1A9 enzymes have yielded negative results on nicotine and cotinine conjugation, most likely related to technical difficulties involved with the UGT expression systems (Ghosheh and Hawes, 2002b; Nakajima et al., 2002b). Taken together, these results suggest that UGT1A4 is the main enzyme catalyzing nicotine and cotinine conjugation, with possibly some involvement of UGT1A9.

There is no direct evidence at this time for the involvement of any specific enzyme in 3′-hydroxycotinine glucuronidation. However, urinary excretion of 3′-hydroxycotinine *O*-glucuronide is correlated with the excretion of NNAL-*O*-glucuronide (Hecht et al., 1999b). NNAL *O*-glucuronidation is catalyzed by UGT1A9 and UGT2B7 (Ren et al., 2000). The excretion of 3′-hydroxycotinine *O*-glucuronide is induced by smoking (Benowitz and Jacob, 2000). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, an aryl hydrocarbon receptor agonist, induces UGT1A9, but does not induce UGT2B7, in human Caco-2 cells (Munzel et al., 1999). Thus, UGT1A9 might be involved in the induction of 3′-hydroxycotinine *O*-glucuronidation by smoking. In microsomes, *N*-glucuronidation of 3′-hydroxycotinine was correlated with *N*-glucuronidation of nicotine and cotinine (Kuehl and Murphy, 2003b). Thus, UGT1A4 and UGT1A9 are probably mediating 3′-hydroxycotinine *N*-glucuronidation. As mentioned before, no 3′-hydroxycotinine *N*-glucuronide has been detected in smokers' urine (Byrd et al., 1994; Kuehl and Murphy, 2003b).

E. Genetic Variations in Nicotine Metabolizing Enzymes

1. CYP2A6 Polymorphisms. Numerous variants of the *CYP2A6* gene have been described in recent years

(Oscarson, 2001; Nakajima et al., 2002a; Xu et al., 2002a). For the most recent update on *P450* gene alleles consult the Human Cytochrome P450 (CYP) Allele Nomenclature Committee website (http://www.imm.ki.se/CYPalleles/). According to this nomenclature, *CYP2A6*1A* denotes the wild-type allele (Table 4).

Most substantial reductions in CYP2A6-mediated metabolism are associated with fully inactive alleles including the *CYP2A6* whole gene deletion alleles (*CYP2A6*4A*, *CYP2A6*4B*, and *CYP2A6*4D*) (Nunoya et al., 1998, 1999a,b; Oscarson et al., 1999a,b) and with an allele (*CYP2A6*2*) containing a single nucleotide change resulting in an inactive enzyme (Yamano et al., 1990; Hadidi et al., 1997). Another single nucleotide polymorphism has also been reported to be inactive due to the protein being unstable (*CYP2A6*5*) (Oscarson et al., 1999a). These alleles encode enzymes that totally lack any enzymatic capacity. Subjects homozygous for *CYP2A6* deletion alleles (*CYP2A6*4*) have very low plasma cotinine levels and urinary excretion of cotinine and 3′-hydroxycotinine after smoking or nicotine administration (Kitagawa et al., 1999; Nakajima et al., 2000, 2001; Kwon et al., 2001; Yang et al., 2001; Xu et al., 2002b; Zhang et al., 2002). We have shown that nonrenal clearance of nicotine in a subject homozygous for *CYP2A6*2* was reduced by 73% compared with the control subjects (Benowitz et al., 1995, 2001); however, nonrenal clearance of cotinine was only reduced by 27%. Two possible explanations can account for these discrepant findings; either the *CYP2A6*2* allele produces an enzyme that has some activity toward cotinine but no activity toward nicotine, or other enzymes are mostly responsible for cotinine clearance in this subject. The first explanation is unlikely since the enzyme encoded by *CYP2A6*2* does not incorporate heme in an in vitro expression system (Yamano et al., 1990).

Several alleles produce enzymes that are functional but have reduced metabolic capacity; many of these have been tested only with coumarin and only in in vitro expression systems (Table 4). *CYP2A6*6* has only one-eighth of the normal coumarin 7-hydroxylation capacity due to a single amino acid substitution (Kitagawa et al., 2001). *CYP2A6*7* is interesting in that its single nucleotide polymorphism causes substantially reduced nicotine metabolism, but reduces coumarin metabolism only slightly, in vivo and in vitro (Ariyoshi et al., 2001; Xu et al., 2002b; Yoshida et al., 2002, 2003). *CYP2A6*8* was previously thought to produce an enzyme with normal catalytic activity (Ariyoshi et al., 2001; Xu et al., 2002b). Recently, however, three individuals with *CYP2A6*8*/*CYP2A6*9* genotype were shown to have low cotinine/nicotine ratios after nicotine administration similar to subjects with the *CYP2A6*4*/*CYP2A6*9* genotype, suggesting that *CYP2A6*8* encodes an enzyme with decreased activity (Yoshida et al., 2003). A single nucleotide polymorphism in the TATA box (TAGA) of the 5′-flanking region of the *CYP2A6* gene (*CYP2A6*9*) reduces transcriptional activity by half; the enzymatic activity is predicted to be similar to the wild-type enzyme because there are no changes to the protein sequence (Pitarque et al., 2001; Kiyotani et al., 2003; Yoshida et al., 2003). Homozygotes for *CYP2A6*9* have lower plasma cotinine/nicotine ratios after nicotine administration compared with wild-type homozygotes (4.3 versus 10.4), indicating that lower expression results in lower metabolic capacity (Yoshida et al., 2003). The *CYP2A6*10* allele contains together both of the single nucleotide changes and resulting amino acid changes, which are found in *CYP2A6*7* and *CYP2A6*8*. This allele has substantially reduced activity in vivo for coumarin and appears inactive for nicotine (Xu et al., 2002b; Yoshida et al., 2002) consistent with unpublished data from our laboratory indicating very slow metabolism of nicotine and markedly reduced conversion of nicotine to cotinine in an individual homozygous for *CYP2A6*10*. *CYP2A6*11* has a single nucleotide polymorphism causing an amino acid change which reduces the V_{max} by half for tegafur, an anticancer drug and a CYP2A6 substrate (Daigo et al., 2002). This allele was detected in a *CYP2A6*4*/*CYP2A6*11* heterozygote having 4-fold higher area under the plasma concentration-time curve of oral tegafur compared with other patients. It has not been tested with nicotine as a substrate. *CYP2A6*12* is a hybrid allele in which the 5′ regulatory region and exons 1 and 2 originate from *CYP2A7* and exons 3–9 originate from *CYP2A6* (Oscarson et al., 2002). This allele causes a 40% reduction in coumarin 7-hydroxylation in vitro in expression studies, and coumarin phenotyping of a *CYP2A6*12* homozygote indicates that it causes reduced CYP2A6 activity in vivo. A recently identified *CYP2A6*17* allele has an amino acid substitution leading to decreased activity toward nicotine in vitro and in vivo (Fukami et al., 2004). A *CYP2A6*17* homozygote had a lower plasma cotinine/nicotine ratio after nicotine administration compared with *CYP2A6*1A* homozygotes (1.8 versus 11.5).

Recent studies have revealed several new polymorphisms in the 5′-flanking region of the *CYP2A6* gene (Kiyotani et al., 2002; Pitarque et al., 2004; Von Richter et al., 2004). Using in vitro transcription assays a common −1013A>G change, found in *CYP2A6*1D* and in some other alleles, reduces transcriptional activity by half, although the effects of these variants on CYP2A6 mRNA levels in hepatic tissues were ambiguous (Pitarque et al., 2004). A −745A>G change in *CYP2A6*1H* allele decreases transcriptional activity slightly by disrupting a CCAAT box, which binds a transcription factor NF-Y (Von Richter et al., 2004). All *CYP2A6*1B* alleles (*CYP2A6*1B1*, *CYP2A6*1B2*, and *CYP2A6*1B3*) and *CYP2A6*7*, *CYP2A6*8*, and *CYP2A6*10* alleles contain a domain swap in the 3′-untranslated region (replacing *CYP2A6* sequence by the corresponding *CYP2A7* sequence) (Oscarson et al., 1999b; Ariyoshi et al., 2000; Kiyotani et al., 2002; Pitarque et al., 2004). The change in the 3′-untranslated region might have relevance in regulating the

TABLE 4
CYP2A6 alleles and their effect on nicotine metabolism

Allele[a]	Nucleotide Changes in the Gene	Effect	Activity Towards Nicotine (or Other Substrates)[b]		References
			In Vivo	In Vitro	
*CYP2A6*1A*	None		Normal	Normal	Yamano et al., 1990
*CYP2A6*1B1* (formerly *CYP2A6*1B*, has also been called *CYP2A6*1E*)	Gene conversion in the 3′ flanking region		Increased?		Nakajima et al., 2001; Yoshida et al., 2003; Pitarque et al., 2004
*CYP2A6*1B2* (formerly *CYP2A6*1B*)	−1013 A → G; gene conversion in the 3′ flanking region				Oscarson et al., 1999b; Ariyoshi et al., 2000; Pitarque et al., 2004
*CYP2A6*1B3* (formerly *CYP2A6*1C*)	−395 G → A; gene conversion in the 3′ flanking region				Kiyotani et al., 2002
*CYP2A6*1C*	See *CYP2A6*1B3*				
*CYP2A6*1D*	−1013 A → G			(Decreased transcription)	Pitarque et al., 2004
*CYP2A6*1E*	See *CYP2A6*1B1*				
*CYP2A6*1F*	5717 C → T				Nakajima et al., 2004
*CYP2A6*1G*	5717 C → T; 5825 A → G				Nakajima et al., 2004
*CYP2A6*1H*	−745 A → G	Disruption of a CCAAT box		(Decreased transcription)	Von Richter et al., 2004
*CYP2A6*1J*	−1013 A → G; −745 A → G				Von Richter et al., 2004
*CYP2A6*1X2*		*CYP2A6* gene duplication	Increased		Rao et al., 2000
*CYP2A6*2*	1799 T → A	L160H	None	(None)	Yamano et al., 1990; Benowitz et al., 2001
*CYP2A6*3*[c]	*CYP2A6*/*CYP2A7* hybrid				Fernandez-Salguero et al., 1995
*CYP2A6*4A-D*[d]	*CYP2A6* deleted	*CYP2A6* deleted	None	(None)	Nunoya et al., 1998; Kitagawa et al., 1999; Nakajima et al., 2000; Kwon et al., 2001; Zhang et al., 2002
*CYP2A6*5*	6582 G → T	G479V	(None)	(None)	Oscarson et al., 1999a
*CYP2A6*6*	1703 G → A	R128Q		(Decreased)	Kitagawa et al., 2001
*CYP2A6*7*	6558 T → C; gene conversion in the 3′ flanking region	I471T	Decreased	Decreased	Ariyoshi et al., 2001; Xu et al., 2002b; Yoshida et al., 2002
*CYP2A6*8*	6600 G → T; gene conversion in the 3′ flanking region	R485L	Decreased		Yoshida et al., 2003
*CYP2A6*9*	−1013 A → G; −48 T → G; 51 G → A	Disruption of the TATA box	Decreased	(Decreased)	Pitarque et al., 2001; Yoshida et al., 2003; Von Richter et al., 2004
*CYP2A6*10*	6558 T → C; 6600 G → T; gene conversion in the 3′-flanking region	I471T; R485L	Decreased		Unpublished data; Yoshida et al., 2002; Xu et al., 2002b
*CYP2A6*11*	3391 T → C	S224P	(Decreased)	(Decreased)	Daigo et al., 2002
*CYP2A6*12*	Exons 1–2 of *CYP2A7* origin; exons 3–9 of *CYP2A6* origin	10 amino acid substitutions	(Decreased)	(Decreased)	Oscarson et al., 2002
*CYP2A6*13*	−48 T → G; 13 G → A	G5R			Kiyotani et al., 2002
*CYP2A6*14*	86 G → A	S29N			Kiyotani et al., 2002
*CYP2A6*15*	−48 T → G; 22 C → T; 2134 A → G	K194E			Kiyotani et al., 2002
*CYP2A6*16*	2161 C → A	R203S			Kiyotani et al., 2002
*CYP2A6*17*	209 C → T; 1779 G → A; 4489 C → T; 5065 G → A; 5163 G → A; 5717 C → T; 5825 A → G	V365M	Decreased	Decreased	Fukami et al., 2004

[a] Nomenclature according to the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (see: http://www.imm.ki.se/CYPalleles/, accessed December 4, 2004).
[b] Activity toward other substrates given in parentheses, if activity toward nicotine is unknown.
[c] *CYP2A6*3* may be an artefact (Oscarson et al., 1998).
[d] *CYP2A6*4A* and *CYP2A6*4C* are identical. Other *CYP2A6*4* alleles are slightly different but all result in deletion of the whole *CYP2A6* gene (Ariyoshi et al., 2002b).

expression level since several regulatory proteins, such as a 43-kDa protein and hnRNPA1 protein, bind to the 3′-untranslated region of CYP2A6 mRNA (Gilmore et al., 2001; Oscarson et al., 2002; Christian et al., 2004). There are indications that CYP2A6 activity might be slightly higher in individuals with *CYP2A6*1B1* alleles compared with individuals with *CYP2A6*1A* alleles (Kwon et al., 2001; Nakajima et al., 2001; Yoshida et al., 2002, 2003). For example, subjects with *CYP2A6*1B1/CYP2A6*4* genotype have a higher cotinine/nicotine ratio than subjects with *CYP2A6*1A/CYP2A6*4* genotype (Yoshida et al., 2003). The effect of the 3′-flanking *CYP2A7* domain swap remains to be clarified in vitro.

There are also genetic *CYP2A6* variants with increased activity. Gene duplication results in *CYP2A6*1XN* allele. Individuals with three functional *CYP2A6* genes (*CYP2A6*1X2/CYP2A6*1*) might have higher metabolic capacity, as evidenced by a lower nicotine/cotinine ratio and a higher intensity of smoking compared with smokers with two or less functional copies of the genes (Rao et al., 2000). Preliminary data from our twin study with nicotine infusions show that subjects with *CYP2A6*1X2/CYP2A6*1* genotype have significantly higher nicotine clearance compared with *CYP2A6*1* homozygotes (21.4 versus 17.3 ml/min/kg) (Benowitz et al., 2002b).

2. Polymorphisms in Other Enzymes. Several polymorphisms have been detected in *CYP2B6*, *CYP2D6*, *CYP2E1*, and *CYP2A13* genes (Bolt et al., 2003; Cascorbi, 2003; Lamba et al., 2003; Zhang et al., 2003; Hesse et al., 2004). CYP2D6 poor metabolizer and extensive metabolizer phenotypes have similar nicotine and cotinine pharmacokinetics (Benowitz et al., 1996). Ultrarapid metabolizer phenotype caused by amplification of *CYP2D6* gene might be associated with increased nicotine metabolism since prevalence of ultrarapid metabolizers in heavy smokers is 4-fold compared with never-smokers, and ultrarapid metabolizers have the lowest nicotine/(cotinine + 3′-hydroxycotinine) ratios (Saarikoski et al., 2000; Caporaso et al., 2001). Detailed analysis on the effect of *CYP2D6* gene amplification on nicotine pharmacokinetics has not been done. *CYP2E1*1D* allele, which is associated with higher enzyme activity after alcohol exposure and in obese subjects (McCarver et al., 1998), is also associated with a higher plasma 3′-hydroxycotinine concentration per cigarette smoked (Howard et al., 2003a). In this study, individuals with at least one *CYP2E1*1D* allele were compared with homozygotes for *CYP2E1*1C* allele after exclusion of subjects with variant *CYP2A6* alleles. *CYP2A13* and *CYP2B6* polymorphisms have not been studied in relation to nicotine metabolism, although there is evidence that some of the variants result in changes in function.

Polymorphisms in aldehyde oxidase gene with functional consequences have not been reported to date (Beedham et al., 2003). Several polymorphisms have been detected in human *FMO3* gene and some of these are associated with the fish-odor syndrome (Cashman and Zhang, 2002). A preliminary report showed markedly impaired urinary excretion of nicotine *N*′-oxide after nicotine chewing gum administration in two siblings with fish-odor syndrome (Ayesh et al., 1988). A few polymorphisms in amine *N*-methyltransferase gene have been reported, but their functional significance is unknown (Thompson et al., 1999). *UGT1A4* gene has alleles with differences in NNAL glucuronidation (Wiener et al., 2004), and one of the *UGT1A9* alleles produces substrate-specific alterations in enzyme activity (Villeneuve et al., 2003). Their effects on nicotine, cotinine, and 3′-hydroxycotinine glucuronidation are unknown.

F. Extrahepatic Nicotine Metabolism

Nicotine is metabolized primarily in the liver. In animals, nicotine metabolism has been shown to occur to a small extent in extrahepatic organs such as lung, kidney, nasal mucosa, and brain (Gorrod and Jenner, 1975; Williams et al., 1990a; Vahakangas and Pelkonen, 1993; Jacob et al., 1997). We are not aware of any recent studies on metabolism of nicotine in human extrahepatic tissues in vitro or in vivo. One early study reports metabolism of nicotine to cotinine and further metabolism of cotinine by human adult, fetal lung, and bronchial epithelium (Boyland and de Kock, 1966).

Expression of CYP2A6 protein is essentially liver-specific (Raunio et al., 2001). Low-level expressions of CYP2A protein in nasal olfactory mucosa, lung, larynx, esophagus, and breast have been detected (Getchell et al., 1993; Degawa et al., 1994; Hellmold et al., 1998; Lechevrel et al., 1999; Hukkanen et al., 2002; Chen et al., 2003b). However, the protein detected might be CYP2A13 since CYP2A6 antibodies cross-react with CYP2A13 (Su et al., 2000). CYP2A13 mRNA levels in nasal mucosa and lung are 5 and 9 times higher than CYP2A6 mRNA levels, respectively (Su et al., 2000). CYP2A6 mRNA has been detected in nasal mucosa, lung, skin, coronary arteries, esophagus, and breast (Hellmold et al., 1998; Su et al., 2000; Janmohamed et al., 2001; Godoy et al., 2002; Borlak et al., 2003).

CYP2B6 protein is expressed in human brain, intestine, kidney, and lung (Gervot et al., 1999; Hukkanen et al., 2002; Miksys et al., 2003). CYP2D6 protein is also expressed in brain (Siegle et al., 2001; Miksys et al., 2002). CYP2E1 protein is expressed in human lung, brain, and esophagus (Lechevrel et al., 1999; Godoy et al., 2002; Hukkanen et al., 2002; Howard et al., 2003b). Interestingly, CYP2B6, CYP2D6, and CYP2E1 proteins are found at higher levels in specific regions from brains of alcoholics who smoke cigarettes compared with nonalcoholics who do not smoke (Miksys et al., 2002, 2003; Howard et al., 2003b).

Aldehyde oxidase is expressed in human liver, lung, kidney, and adrenal gland (Moriwaki et al., 2001). Human FMO3 is mostly expressed in liver, but also in brain, especially in substantia nigra (Cashman and Zhang, 2002). Low levels of FMO3 have been detected in

human kidney (Krause et al., 2003). The expression of amine *N*-methyltransferase is highest in human thyroid, adrenal gland, and lung (Thompson et al., 1999). UGT1A4 is detected in liver, colon, and gastric and biliary tissue but not in respiratory tract or esophagus (Strassburg et al., 1998; Zheng et al., 2002). The highest expression of UGT1A9 is detected in kidney, followed by small intestine, liver, stomach, esophagus, testis, ovary, and mammary gland (McGurk et al., 1998; Albert et al., 1999; Strassburg et al., 1999). However, it is not expressed in the respiratory tract (Zheng et al., 2002). No nicotine or cotinine glucuronidation has been detected in microsomes from lung, kidney, or gastrointestinal tract (Ghosheh and Hawes, 2002a).

It can be concluded that extrahepatic nicotine metabolism in humans is probably of little importance for systemic nicotine clearance. Considering mRNA and protein expression levels in extrahepatic tissues, CYP2A13 may metabolize nicotine in the respiratory tract, especially in nasal mucosa, and CYP2B6, CYP2D6, and CYP2E1 may metabolize nicotine in the brain. Extrahepatic nicotine metabolism clearly needs more research in humans. Covalent binding of reactive metabolites to macromolecules may have pharmacologic or toxicologic significance, and extrahepatic metabolism may influence nicotine concentrations in organs where nicotine exerts pharmacologic effects.

G. Factors Influencing Nicotine Metabolism

There is considerable interindividual variability in the rate of elimination of nicotine and cotinine in people (Benowitz et al., 1982a). Besides genetic variations discussed above, a number of factors that might explain interindividual variability have been studied.

1. Physiological Influences.

a. Diet and Meals. An implication of the high degree of hepatic extraction is that clearance of nicotine should be dependent upon liver blood flow. Thus, physiological events, such as meals, posture, exercise, or drugs perturbing hepatic blood flow, are predicted to affect the rate of nicotine metabolism. We have found that meals consumed during a steady-state infusion of nicotine result in a consistent decline in nicotine concentrations, the maximal effect seen 30 to 60 min after the end of a meal (Lee et al., 1989; Gries et al., 1996). Hepatic blood flow increases about 30% and nicotine clearance increases about 40% after a meal.

Menthol is widely used as a flavorant in foods, mouthwash, toothpaste, and cigarettes. Menthol cigarette smoking has been associated with longer cotinine half-life in women (Ahijevych et al., 2002). Recently, a moderate inhibition of CYP2A6-mediated nicotine metabolism in human liver microsomes by menthol and various related compounds was reported (MacDougall et al., 2003). This is supported by a crossover study in people, showing that mentholated cigarette smoking significantly inhibits metabolism of nicotine to cotinine and nicotine glucuronidation when compared with smoking nonmentholated cigarettes (Benowitz et al., 2004a). The effect of pure menthol on nicotine and cotinine pharmacokinetics in vivo is yet to be studied.

Grapefruit juice has a strong inhibitory effect on CYP3A4 leading to clinically important interactions (Bailey et al., 1991; Dresser et al., 2000). Grapefruit juice inhibits also CYP2A6 as evidenced by the inhibition of coumarin metabolism in vivo (Merkel et al., 1994; Runkel et al., 1997). The inhibition occurs when a 300- to 1000-ml dose of grapefruit juice is consumed simultaneously with coumarin. The use of wheatgrass juice as a part of uncooked vegan diet has also been shown to inhibit coumarin metabolism (Rauma et al., 1996). The effects of grapefruit juice and wheatgrass juice on nicotine metabolism have not been studied. Consumption of watercress enhances the formation of nicotine glucuronide, cotinine glucuronide, and 3′-hydroxycotinine glucuronide in smokers (Hecht et al., 1999b). Watercress has no effect on coumarin metabolism or the excretion of nicotine, cotinine, and 3′-hydroxycotinine in smokers (Hecht et al., 1999b; Murphy et al., 2001). Thus, watercress may induce some UGT enzymes involved in nicotine metabolism, but has no effect on CYP2A6-mediated nicotine metabolism.

b. Age. Clearance of nicotine is decreased in elderly (age >65) compared with adults (Molander et al., 2001). Total clearance was lower by 23% and renal clearance lower by 49% in the elderly compared with young adults. Lower CYP2A6-mediated metabolism in older subjects has also been demonstrated with coumarin (Sotaniemi et al., 1996). Lower nicotine metabolism in the elderly may be contributed to by reduced liver blood flow, as no decrease in CYP2A6 protein levels or nicotine metabolism in liver microsomes due to age has been detected (Shimada et al., 1994; Messina et al., 1997; Baker et al., 2001). No differences in steady-state nicotine plasma levels or estimated plasma clearance values were detected in three age groups (18–39, 40–59, and 60–69 years) using patches with same nicotine content (Gourlay and Benowitz, 1996). Volume of distribution of nicotine is lower in older subjects due to decrease in lean body mass (Molander et al., 2001).

Neonates have diminished nicotine metabolism as demonstrated by 3 to 4 times longer nicotine half-life in newborns exposed to tobacco smoke compared with adults (Dempsey et al., 2000). Cotinine half-life is reported to be similar in neonates, older children, and adults in two studies (Leong et al., 1998; Dempsey et al., 2000), but two other studies found that half-life of urine cotinine was about 3 times longer in children less than 1 year old compared with the cotinine half-life in adults (Etzel et al., 1985; Collier et al., 1994). Urine cotinine half-life is prone to be influenced by variations in urine volume and excretion of creatinine. The study by Dempsey et al. (2000) was the only one in which half-life of cotinine was calculated based on both the blood and urine cotinine concentrations. In that study, both the

blood and urine half-life were similar to adult values supporting the notion that neonates have the same cotinine half-life as older children and adults. Why then does nicotine have much longer half-life in neonates than in adults, whereas cotinine half-life is essentially the same in newborns and adults? This might be partly explained by differing sensitivities of nicotine and cotinine clearances to changes in hepatic blood flow. As a drug with high extraction ratio, the clearance of nicotine is influenced by changes in hepatic blood flow, whereas clearance of cotinine with low extraction ratio is more dependent on changes in intrinsic clearance, i.e., amount and activity of metabolic enzymes. Studies in newborn animals, mainly sheep, have shown that hepatic blood flow is low immediately after delivery because of the loss of the umbilical venous blood supply and the patency of ductus venosus (Gow et al., 2001). Hepatic blood flow (milliliters per minute per gram of liver) rises to adult levels within the first week, due to increase in blood flow in portal vein and gradual closure of ductus venosus, which is complete by day 18 in human neonates. This would mean that nicotine clearance should rise and nicotine half-life shorten within the first couple of weeks as hepatic blood flow increases. There are not yet experimental data to support this hypothesis. Another explanation could be that nicotine and cotinine are metabolized mainly by enzymes other than CYP2A6 in neonates. However, neonates have only slightly lower amounts of CYP2A6, CYP2D6, and CYP2E1 protein in liver microsomes, whereas CYP2B6 amount is clearly diminished in neonates compared with adults and older children (Tateishi et al., 1997).

No significant differences in CYP2A6-mediated metabolism have been demonstrated between adults and older children. Children aged 6 to 13 years have similar metabolism of coumarin, children aged 7 to 16 years have similar caffeine ratio as an index of CYP2A6 activity, and children aged 4 to 11 years have similar cotinine half-life as adults (Willers et al., 1995; Pasanen et al., 1997; Krul and Hageman, 1998). No apparent differences are seen in coumarin metabolism or CYP2A6 protein levels in liver microsomes in children more than 2 years old compared with adults (Koenigs et al., 1997; Messina et al., 1997; Tateishi et al., 1997).

c. Chronopharmacokinetics of Nicotine. During sleep, hepatic blood flow declines and nicotine clearance falls correspondingly. Blood nicotine levels during constant infusion rise at night. Nicotine clearance varies by approximately 17% (from peak to through) with minimum between 6:00 PM and 3:00 AM. Thus, the day/night variation and meal effects of nicotine clearance result in circadian variations in plasma concentrations during constant dosing of nicotine (Gries et al., 1996). Menstrual cycle (follicular phase versus luteal phase) has no effect on nicotine and cotinine pharmacokinetics in healthy nonsmoking women (Hukkanen et al., 2005). Seasonal changes in nicotine metabolism have not been studied.

d. Gender-Related Differences in Nicotine Metabolism.

i. Differences between Men and Women. Varying results from previous small-scale studies comparing nicotine and cotinine clearance in men and women have been reported. In a small group of habitual heavy cigarette smokers, the clearance of nicotine corrected for body weight was found to be significantly higher in men than women (Benowitz and Jacob, 1984). In a later study, we found that clearance normalized for body weight was similar in men and women, although total clearance was lower in women compared with men because on average they weigh less (Benowitz and Jacob, 1994). A study with infusions of nicotine and cotinine in smokers showed significantly shorter half-life in women compared with men for cotinine, but not for nicotine (Benowitz et al., 1999). In another small-scale study by Prather et al. (1993) with a single application of a nicotine patch, women had a significantly higher elimination rate constant value compared with men, suggesting quicker elimination of nicotine in women.

Results from a recently completed large-scale (N = 290) twin study with intravenous infusions of both nicotine and cotinine clearly show that nicotine and cotinine clearances are higher in women compared with men, and oral contraceptive use further accelerates nicotine and cotinine clearances in women (Benowitz et al., 2004b). Nicotine clearance and cotinine clearance were 13 and 26% higher, respectively, in women not using oral contraceptives compared with men. Oral contraceptive use induced nicotine and cotinine clearances by 30 and 33%, respectively, compared with women not using oral contraceptives. The gender difference was also detected in a recent study on smokers (N = 400) showing that ratio of nicotine/(cotinine + 3′-hydroxycotinine) in 24-h urine collection is significantly lower in women indicating faster metabolism in women compared with men (Zeman et al., 2002). Another slightly smaller study (N = 261) showed no gender difference in nicotine/(cotinine + 3′-hydroxycotinine) ratio in smokers (Caporaso et al., 2001). Subjects with defective *CYP2A6* alleles were excluded in the former study but not in the latter study. Inclusion of subjects with dysfunctional alleles reduces the statistical power to detect gender differences and may bias the results if these alleles are not equally distributed between men and women in the study.

A tendency for higher metabolism in women compared with men has been detected in most (six of seven studies) of the phenotyping studies using coumarin 7-hydroxylation or conversion of caffeine metabolite 1,7-dimethylxanthine to 1,7-dimethyluric acid as a measure of CYP2A6 activity (Rautio et al., 1992; Iscan et al., 1994; Sotaniemi et al., 1996; Krul and Hageman, 1998; Nowell et al., 2002; Ujjin et al., 2002; Xu et al., 2002c). Two of these studies showed a statistically significant

difference; 17% higher 3-h urinary excretion of 7-hydroxycoumarin in females compared with males was reported by Ujjin et al. (2002), and the study by Iscan et al. (1994) showed 11% higher 7-hydroxycoumarin excretion in women compared with men in a 2-h coumarin test. A third study showed significantly higher activity in women compared with men only in a subpopulation with lower coumarin hydroxylase activity (Xu et al., 2002c). Significant gender differences have not been detected in the amount or activity of CYP2A6 in liver microsomes in several studies with a small number of samples (Pearce et al., 1992; Shimada et al., 1994; Bourrie et al., 1996; Chauret et al., 1997; Baker et al., 2001; Schmidt et al., 2001).

A tendency for slower glucuronidation of nicotine and cotinine in women compared with men was reported in a study using human liver microsomes (Ghosheh and Hawes, 2002a). However, no gender differences in glucuronidation were detected in one study measuring urinary excretion of glucuronides of nicotine, cotinine, and 3′-hydroxycotinine after infusion of nicotine and cotinine (Benowitz et al., 1999).

ii. Pregnancy and Menstrual Cycle. Pregnancy has a marked inducing effect in nicotine and especially cotinine clearance. Clearance is increased by 60 and 140% for nicotine and cotinine, respectively, in pregnancy compared with postpartum (Dempsey et al., 2002). Nicotine is a rapidly cleared drug with a high affinity for CYP2A6, and its rate of clearance is primarily controlled by hepatic blood flow, whereas the rate of cotinine clearance is primarily determined by the activity of metabolizing enzymes in liver. The finding that in pregnancy cotinine clearance is increased more than nicotine clearance indicates that increase in clearance is most likely caused by induction of CYP2A6 and not by an increase in hepatic blood flow. A study comparing women during pregnancy and again postpartum found that mean salivary cotinine concentration per cigarette was higher when not pregnant (3.5 versus 9.9 ng/ml) consistent with higher cotinine clearance during pregnancy (Rebagliato et al., 1998). Pregnant smokers had a substantially lower level of serum nicotine than expected when standardized for their nicotine intake compared with population-based values (Selby et al., 2001). Nicotine and cotinine glucuronidation is induced by pregnancy, whereas 3′-hydroxycotinine glucuronidation is not (Dempsey et al., 2002). Menstrual cycle (follicular phase versus luteal phase) has no effect on nicotine and cotinine pharmacokinetics in healthy nonsmoking women (Hukkanen et al., 2005).

The above-mentioned results show that sex has substantial effects on nicotine and cotinine metabolism. Higher metabolism of nicotine and cotinine is detected in women compared with men, in users of oral contraceptives compared with women not using oral contraceptives, and in pregnant women compared with the same subjects postpartum. Furthermore, the inducing effect has a dose-response relationship; gender differences are relatively small, oral contraceptive use further induces metabolism in women, and pregnancy shows the most striking induction compared with postpartum. Changes in clearance appear to be related to the amount of sex hormones present; women have higher concentrations of estrogens and progesterone than men do, oral contraceptive users have higher concentrations of these hormones than women not using oral contraceptives, and pregnancy results in the highest concentrations of circulating sex hormones. These results suggest that CYP2A6 activity is induced by sex hormones; however, supporting experimental in vitro data are still lacking.

2. Pathological Conditions. Certain diseases have been shown to affect CYP2A6-mediated metabolism. Hepatitis A markedly reduces coumarin metabolism in adults and children (Pasanen et al., 1997), whereas liver fluke parasite infection induces coumarin metabolism (Satarug et al., 1996). Acute alcohol ingestion is without effect on coumarin metabolism (Rautio et al., 1994), whereas patients with alcoholic liver disease have reduced metabolism of coumarin detected both in liver biopsy samples and in vivo (Kratz, 1976; Pelkonen et al., 1985; Sotaniemi et al., 1995). Although the total metabolism by CYP2A6 is reduced in patients with alcoholic liver disease and viral hepatitis, CYP2A6 expression seems to be induced in areas of the liver immediately next to fibrotic and inflamed areas as shown with in situ hybridization and immunohistochemistry (Palmer et al., 1992; Kirby et al., 1996; Niemela et al., 2000). Increased CYP2A6 expression is also found in areas adjacent to hepatocellular carcinoma (Raunio et al., 1998). Consistent with the finding that CYP2A6-mediated metabolism is impaired in alcoholic liver disease, cotinine levels stratified by cigarette consumption were recently shown to be higher in smokers with alcoholic cirrhosis compared with healthy smokers (Langmann et al., 2000).

Kidney failure not only decreases renal clearance of nicotine and cotinine but also metabolic clearance of nicotine (Molander et al., 2000). Metabolic clearance of nicotine is reduced by 50% in subjects with severe renal impairment compared with healthy subjects. It is speculated that accumulation of uremic toxins may inhibit CYP2A6 activity or down-regulate CYP2A6 expression in liver. Hepatic metabolism of several drugs is reduced in kidney failure, mainly via down-regulation of P450 enzymes and/or inhibition of transporters (Nolin et al., 2003).

Trimethylaminuria (fish-odor syndrome) is caused by genetic defect in FMO3 enzyme leading to deficient *N*-oxygenation of dietary trimethylamine (Cashman and Zhang, 2002). A preliminary report of two siblings with fish-odor syndrome showed impaired urinary excretion of nicotine *N*′-oxide when compared with normal volunteers after nicotine chewing gum administration (Ayesh et al., 1988).

3. Medications.

a. Inducers. A few drugs have been shown to induce CYP2A6 in human primary hepatocyte culture. These include prototypical inducers rifampicin, dexamethasone, and phenobarbital, although there is wide interindividual variability in response (Maurice et al., 1991; Dalet-Beluche et al., 1992; Mattes and Li, 1997; Meunier et al., 2000; Robertson et al., 2000; Rae et al., 2001; Edwards et al., 2003; Madan et al., 2003). Pyrazole is an inducer of CYP2A6 in vitro (Donato et al., 2000). Rifampicin was recently shown to inhibit CYP2A6 activity as measured by coumarin 7-hydroxylase with an IC_{50} of 39 μM (Xia et al., 2002). That might partly explain the highly variable results on CYP2A6 induction detected with the 50 μM concentration of rifampicin usually used in induction studies.

There is some evidence for the induction of CYP2A6 in vivo by phenobarbital and other anticonvulsant drugs. Coumarin phenotyping shows increased metabolism in epileptic patients treated with carbamazepine, clonazepam, phenobarbital, and/or phenytoin (Sotaniemi et al., 1995). Two-day treatment with phenobarbital (100 mg/day p.o.) prior to a liver biopsy resulted in induction of metabolism of nicotine to cotinine in hepatocytes (Kyerematen et al., 1990a). Liver microsomes from phenobarbital-treated patients have higher amounts of CYP2A6 protein than microsomes from untreated patients (Yamano et al., 1990; Cashman et al., 1992). Rifampicin treatment (450 mg/day p.o.) for 6 days had no effect on CYP2A6 activity measured as coumarin hydroxylation (Rautio et al., 1994).

As mentioned earlier, preliminary results from our twin study show that nicotine and cotinine clearances are higher in women using oral contraceptives compared with women not using oral contraceptives (Benowitz et al., 2004b). Oral contraceptive use induced nicotine and cotinine clearances by 30 and 33%, respectively. A previous small-scale study with caffeine phenotyping of CYP2A6 showed a 22% increase in CYP2A6 activity in oral contraceptive users compared with women not using contraceptives (Krul and Hageman, 1998). The difference was statistically nonsignificant due to lack of power ($N = 5$ per group). We know of no in vitro studies exploring the inducing effects of sex hormones on CYP2A6.

Induction of P450 enzymes by rifampicin is mediated via pregnane X receptor (PXR), whereas phenobarbital acts via both PXR and constitutive androstane receptor (CAR) (Moore et al., 2000). A recently identified selective CAR ligand 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime was shown to induce CYP2A6 in human primary cultures of hepatocytes, whereas rifampicin was without effect in that study (Maglich et al., 2003). This suggests that induction of CYP2A6 is mainly mediated by CAR and not by PXR. Dexamethasone induces P450 enzymes by three different routes: direct glucocorticoid receptor-mediated induction in nanomolar concentrations (CYP2C8, CYP2C9, CYP2C19, and CYP3A5), direct PXR-mediated induction in micromolar concentrations (CYP3A4), and indirect mechanism in nanomolar concentrations where dexamethasone induces CAR and PXR expression via glucocorticoid receptor and thus indirectly induces P450 expression (CYP2C8, CYP2C9, CYP2B6, and CYP3A4) (Pascussi et al., 2000a,b; Gerbal-Chaloin et al., 2001, 2002; Pascussi et al., 2001; Chen et al., 2003a; Hukkanen et al., 2003; Wang et al., 2003a). It is currently unknown which mechanism is responsible for the induction of CYP2A6 by dexamethasone. Mouse CAR is activated in vitro by estrogens and repressed by progesterone and testosterone (Kawamoto et al., 2000; Ueda et al., 2002). However, human CAR seems not to be affected by these sex hormones in the experimental design used (transfection experiments in HepG2 cell line). Interestingly, CYP2A6 mRNA was recently shown to be expressed in much higher levels in estrogen receptor α-positive breast tumors compared with estrogen receptor α-negative tumors (Bieche et al., 2004). Possible induction of human CYP2A6 by sex hormones needs to be studied more thoroughly in vitro to elucidate the mechanisms of induction.

A recent study ($N = 197$, four subjects homozygous for *CYP2A6*4* excluded) with coumarin phenotyping of CYP2A6 found an intriguing association between cadmium exposure and CYP2A6 activity (Satarug et al., 2004). High levels of urine cadmium excretion were correlated with high coumarin 7-hydroxylase activity. In a multiple regression analysis, urine cadmium excretion accounted for 25% of the variation in CYP2A6 activity, whereas gender (higher activity in women compared with men) explained 3% of the variation. CYP2A5, a mouse ortholog of CYP2A6, is induced in vivo by cadmium chloride administration via transcription factor Nrf2 (NF-E2-related factor 2) mediated mechanism (Abu-Bakar et al., 2004). Nrf2 is an important transcription factor in the regulation of several phase II enzymes (Nguyen et al., 2003).

b. Inhibitors. Several compounds are inhibitors of CYP2A6-mediated nicotine metabolism in vitro, including methoxsalen (8-methoxypsoralen), tranylcypromine, tryptamine, coumarin, and neomenthyl thiol (Nakajima et al., 1996b; Zhang et al., 2001; Le Gal et al., 2003; MacDougall et al., 2003). Other inhibitors of coumarin 7-hydroxylation such as pilocarpine, metyrapone, *R*-(+)-menthofuran, β-nicotyrine, naphthalene, 4,4′-dipyridyl disulfide, 2-(*p*-tolyl)-ethylamine, nootkatone, several azole antifungals, and rifampicin would also be expected to inhibit nicotine *C*-oxidation (Pelkonen et al., 2000; Tassaneeyakul et al., 2000; Fujita and Kamataki, 2001; Xia et al., 2002; Asikainen et al., 2003; Rahnasto et al., 2003; Denton et al., 2004). Raloxifene is a potent inhibitor of aldehyde oxidase and it has been shown to inhibit the formation of cotinine from nicotine-$\Delta^{1'(5')}$-iminium ion in human liver cytosol (Obach, 2004).

Only methoxsalen (used in the photochemotherapy of psoriasis) and tranylcypromine (a monoamine oxidase inhibitor) have been demonstrated to inhibit nicotine and coumarin metabolism in people (Maenpaa et al., 1994; Kharasch et al., 2000; Sellers et al., 2000, 2003a; Tyndale and Sellers, 2001). These compounds are only moderately specific for CYP2A6; methoxsalen is also a potent inhibitor of CYP1A2, and tranylcypromine inhibits CYP2B6 and CYP2E1 (Taavitsainen et al., 2001; Zhang et al., 2001). Methoxsalen reduces first-pass metabolism of oral nicotine, decreases clearance of subcutaneously administered nicotine, and decreases urinary levels of 3′-hydroxycotinine in smokers (Sellers et al., 2000, 2003a). Tranylcypromine has been shown to reduce first-pass metabolism of oral nicotine (Tyndale and Sellers, 2001). Because smokers smoke at least in part to maintain desired levels of nicotine in the brain, decreased metabolism and higher concentration of nicotine result in a reduction in the number of cigarettes smoked (Sellers et al., 2000). Also, as CYP2A6 is involved in the activation of carcinogenic NNK, inhibition of CYP2A6 routes the metabolism of NNK toward the inactive NNAL-glucuronide (Sellers et al., 2003a). Thus, CYP2A6 inhibitors might be of use in the reduction of smoking, decreasing the exposure to carcinogenic metabolites and possibly reducing the risk of cancer, and enhancing the efficacy of nicotine replacement therapies (Sellers et al., 2003b).

A preliminary report has been published on the effect of FMO3 inhibition on nicotine *N*′-oxide formation. Methimazole, an antithyroid agent and FMO3 inhibitor, was shown to reduce nicotine *N*′-oxide excretion after nicotine administration with chewing gum (Cholerton et al., 1988).

4. Smoking.

a. Inhibiting Effect on Nicotine Clearance. Cigarette smoking itself influences the rate of metabolism of nicotine. Cigarette smoking is known to accelerate the metabolism of some drugs, especially the ones primarily metabolized by CYP1A2 (Zevin and Benowitz, 1999). However, we found that the clearance of nicotine was significantly slower in cigarette smokers compared with nonsmokers (Benowitz and Jacob, 1993). In support of this observation are two crossover studies comparing the clearance of nicotine in the same subjects when smoking compared with not smoking. After 4 days of smoking abstinence, nicotine clearance was increased by 14% (Benowitz and Jacob, 2000), and after 7 days of abstinence, nicotine clearance was 36% higher (Lee et al., 1987) when compared with overnight abstinence from cigarettes. Also studies with coumarin support the reducing effect of smoking on CYP2A6-mediated metabolism (Iscan et al., 1994; Poland et al., 2000).

These studies suggest that there are substance(s) in tobacco smoke, as yet unidentified, that inhibit the metabolism of nicotine. Because nicotine and cotinine are metabolized by the same enzyme, the possibility that cotinine might be responsible for the slowed metabolism of nicotine in smokers was examined. In a study in which nonsmokers received an intravenous infusion of nicotine with and without pretreatment with high doses of cotinine, there was no effect of cotinine on the clearance of nicotine (Zevin et al., 1997). Also carbon monoxide at levels and in a pattern similar to those experienced during smoking had no effect on nicotine and cotinine clearance (Benowitz and Jacob, 2000).

Recently, β-nicotyrine, a minor tobacco alkaloid, was shown to effectively inhibit CYP2A6 in vitro (Denton et al., 2004). Thus, β-nicotyrine is one candidate for the search on the inhibiting compound in tobacco smoke. Another possibility is that reduced nicotine clearance is due to down-regulation of CYP2A6 expression and not due to inhibition. Tyndale and coworkers have demonstrated that administration of nicotine for 21 days to monkeys in vivo decreases CYP2A6 activity (nicotine metabolism) by down-regulating CYP2A6 mRNA and protein in liver (Schoedel et al., 2003). Interestingly, expression of both CYP2A and CYP3A5 mRNAs are markedly reduced in human pulmonary tissues in smokers compared with nonsmokers (Crawford et al., 1998; Hukkanen et al., 2003). The mechanisms of the down-regulation are currently unknown.

b. Inducing Effect of Smoking on Glucuronidation. We have recently shown that excretion of 3′-hydroxycotinine *O*-glucuronide is induced by smoking when compared with not smoking studied with a crossover design (Benowitz and Jacob, 2000). The extent of nicotine and cotinine *N*-glucuronidation was not significantly affected by smoking. Smoking is known to induce glucuronidation of some drugs, such as propranolol and oxazepam (Liston et al., 2001). The enzyme(s) responsible for glucuronidation of 3′-hydroxycotinine is unknown. However, urinary excretion of 3′-hydroxycotinine *O*-glucuronide is correlated with the excretion of NNAL-*O*-glucuronide (Hecht et al., 1999b), which is formed by UGT1A9 and UGT2B7 (Ren et al., 2000). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, an aryl hydrocarbon receptor agonist, induces UGT1A9, but does not induce UGT2B7, in human Caco-2 cells (Munzel et al., 1999). Thus, UGT1A9 might be the inducible component of 3′-hydroxycotinine *O*-glucuronidation.

5. Racial and Ethnic Differences. Racial differences in nicotine and cotinine metabolism have been observed. We compared nicotine and cotinine metabolism in blacks and whites of similar age and body weight ($N = 108$) (Perez-Stable et al., 1998; Benowitz et al., 1999). The total and nonrenal clearance of cotinine was significantly lower in blacks versus whites (total clearance 0.57 versus 0.76 ml/min/kg). Also the fractional clearance of nicotine to cotinine and the metabolic clearance of nicotine to cotinine were lower in blacks. The clearance of nicotine tended to be lower in blacks versus whites (18.1 versus 20.5 ml/min/kg), but this difference was not significant. Excretion of nicotine and cotinine glucuronides was lower in blacks, whereas excretion of

3′-hydroxycotinine glucuronide was similar in both groups. Nicotine and cotinine glucuronidation appeared to be polymorphic in blacks, with evidence of slow and fast *N*-glucuronide formers. The distribution of glucuronidation was unimodal in whites. Polymorphic pattern of cotinine glucuronidation in blacks has been detected in other studies (de Leon et al., 2002). Slower metabolism of cotinine explains in part the higher cotinine levels per cigarette detected in blacks compared with whites (Wagenknecht et al., 1990; English et al., 1994; Ahijevych and Gillespie, 1997; Caraballo et al., 1998). One possible explanation for the slower cotinine metabolism in blacks is the significantly higher proportion of menthol cigarette smokers in blacks compared with whites (69% versus 22% in the general U.S. population, 76 versus 9% in our study) (Benowitz et al., 1999; Giovino et al., 2004). As discussed earlier, menthol cigarette smoking inhibits nicotine oxidation and glucuronidation (Benowitz et al., 2004a). A recently identified *CYP2A6*17* allele with reduced activity toward nicotine seems to be specific for blacks (Fukami et al., 2004). No significant differences have been detected in other *CYP2A6* genotype frequencies between blacks and whites (Paschke et al., 2001; Schoedel et al., 2004).

We have compared nicotine and cotinine metabolism among Chinese-Americans, Latinos, and whites ($N = 131$) (Benowitz et al., 2002a). Chinese-Americans had the lowest total and nonrenal clearance of nicotine and cotinine and metabolic clearance of nicotine via the cotinine pathway. Also nicotine intake per cigarette was lower in Chinese-Americans compared with Latinos and whites. No significant differences in nicotine and cotinine metabolism or nicotine intake were detected between Latinos and whites. Glucuronidation of nicotine and metabolites did not differ between the groups. *CYP2A6* genotypes were not analyzed in the study, but in other studies, Chinese have been shown to have markedly higher frequencies of dysfunctional or reduced activity *CYP2A6* alleles compared with Caucasians (combined frequencies of null and reduced activity alleles 31% in Chinese versus 13% in Caucasians) (Table 5). In particular, the *CYP2A6*4* (a null allele) and *CYP2A6*9* (reduced expression) alleles are frequent in Chinese with allele frequencies of 7.6% and 15.7%, respectively (Pitarque et al., 2001; Wang et al., 2003b).

In a recent study, Japanese male smokers were shown to have higher plasma nicotine and lower cotinine levels after overnight tobacco abstinence compared with American males, whereas this difference was not detected in women (Domino et al., 2003). The lower cotinine levels in Japanese have also been detected in other studies (Muranaka et al., 1988). In a study with liver microsomes, a considerable fraction of Japanese samples (8/30) had no detectable CYP2A6 protein and coumarin hydroxylase activity, whereas all of the Caucasian samples (30/30) had

TABLE 5
CYP2A6 allele frequency distribution in various populations[a]

Allele	African-Americans	Caucasians	Chinese	Japanese	Koreans	Native Canadians	Sri Lankans	Thais	References
	%	%	%	%	%	%	%	%	
*CYP2A6*1B*[b]	13.0	33.5	51.3	42.5	37.1	55.0	31.5	39.7	Ariyoshi et al., 2002a; Yoshida et al., 2002; Ujjin et al., 2002; Topcu et al., 2002; Schoedel et al., 2004
*CYP2A6*1X2*	0	0.7	0.4	0	0.2	0			Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*2*	1.1	2.2	0	0	0	0			Kitagawa et al., 2001; Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*3*[c]	0	0	0	0	0				Oscarson et al., 1999a; Kitagawa et al., 2001; Paschke et al., 2001; Yoshida et al., 2002
*CYP2A6*4*	1.9	1.2	7.6	20.4	11.0	1.0	9.6	7.8	Ariyoshi et al., 2002a; Topcu et al., 2002; Yoshida et al., 2002; Ujjin et al., 2002; Wang et al., 2003b; Schoedel et al., 2004
*CYP2A6*5*	0	0.1	0.5	0	0.5	0.5			Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*6*	0	0	0	0.2	0	0			Kitagawa et al., 2001; Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*7*	0	0.3	3.1	6.5	3.6	0			Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*8*	0	0.1	3.6	2.2	1.4	0			Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*9*	7.1	7.1	15.7	21.3	22.3	15.5			Pitarque et al., 2001; Yoshida et al., 2003; Schoedel et al., 2004
*CYP2A6*10*	0	0	0.4	1.1	0.5	0			Yoshida et al., 2002; Schoedel et al., 2004
*CYP2A6*11*		0		0.6	0.7				Daigo et al., 2002; Nakajima et al., 2002a; Kiyotani et al., 2003
*CYP2A6*12*	0.4	2.0	0	0.8		0.5			Schoedel et al., 2004
*CYP2A6*13*		0		1.5					Kiyotani et al., 2002
*CYP2A6*14*		3.6		0					Kiyotani et al., 2002
*CYP2A6*15*		0		1.5					Kiyotani et al., 2002
*CYP2A6*16*		3.6		0					Kiyotani et al., 2002
*CYP2A6*17*	9.4	0		0	0				Fukami et al., 2004

[a] As the data are gathered from several studies with differing methodologies, the reader is advised to interpret them with caution. If more than one study reports allele frequencies for a given allele in an ethnic group, the percentage shown is selected taking into account the technique used, the scope of the genotypes studied, and the number of subjects.
[b] Since only the domain swap in the 3′-untranslated region was investigated in these studies, more precise nomenclature (e.g. *CYP2A6*1B1*) cannot be used.
[c] *CYP2A6*3* may be an artefact (Oscarson et al., 1998).

measurable protein and activity levels (Shimada et al., 1996). These results are consistent with the higher frequencies of null and reduced activity alleles of *CYP2A6* in Japanese compared with Caucasians. Combined frequencies of null and reduced activity alleles are 53, 40, and 13% in Japanese, Korean, and Caucasian individuals, respectively (Table 5). *CYP2A6*4* and *CYP2A6*9* alleles are the most frequent dysfunctional alleles in Japanese and Korean populations with allele frequencies of 20.4 and 21.3% (Japanese) and 11.0 and 22.3% (Koreans), respectively (Ariyoshi et al., 2002; Yoshida et al., 2002, 2003).

VII. Excretion

A. Renal Excretion

Nicotine is excreted by glomerular filtration and tubular secretion, with variable reabsorption depending on urinary pH. With uncontrolled urine pH, renal clearance averages about 35 to 90 ml/min, accounting for the elimination of about 5% of total clearance (Table 2). In acid urine, nicotine is mostly ionized and tubular reabsorption is minimized; renal clearance may be as high as 600 ml/min (urinary pH 4.4), depending on urinary flow rate (Benowitz and Jacob, 1985). In alkaline urine, a larger fraction of nicotine is unionized, allowing net tubular reabsorption with renal clearance as low as 17 ml/min (urine pH 7.0).

In vitro studies in pig kidney-derived LLC-PK_1 cells have shown that there are distinct transport systems for both basolateral and apical uptake of nicotine (Takami et al., 1998). Basolateral uptake was inhibited by cotinine, tetraethylammonium, cimetidine, and quinidine, and apical uptake was inhibited by quinidine and levofloxacin (but not by cotinine, tetraethylammonium, and cimetidine), suggesting that the transport of nicotine may be mediated by specific transport systems rather than by passive diffusion alone. Cimetidine decreases renal clearance of nicotine by 47% in nonsmoking volunteers (Bendayan et al., 1990). This is consistent with the inhibition of basolateral uptake by cimetidine detected in vitro. Mecamylamine reduces renal clearance of nicotine in smokers dosed with intravenous nicotine when urine is alkalinized, but not when urine is acidified (Zevin et al., 2000a). Nicotine has been shown to inhibit human organic cation transporters OCTN1, OCT2 and its functionally distinct splicing variant OCT2-A, as well as rat OCTN1, OCT1, and OCT2 in vitro (Urakami et al., 1998, 2002; Yabuuchi et al., 1999; Wu et al., 2000) suggesting that nicotine may be a substrate for organic cation transporters. In contrast, nicotine is not a P-glycoprotein substrate (Fukada et al., 2002).

Renal clearance of cotinine is much less than glomerular filtration rate (Benowitz et al., 1983b). Since cotinine is not appreciably protein bound, this indicates extensive tubular reabsorption. Renal clearance of cotinine can be enhanced by up to 50% with extreme urinary acidification. Cotinine excretion is less influenced by urinary pH than nicotine because it is less basic and, therefore, is primarily in the unionized form within the physiological pH range. As is the case for nicotine, the rate of excretion of cotinine is influenced by urinary flow rate. Renal excretion of cotinine is a minor route of elimination, averaging about 12% of total clearance. In contrast, 100% of nicotine *N*′-oxide and 63% of 3′-hydroxycotinine are excreted unchanged in the urine (Park et al., 1993; Benowitz and Jacob, 2001).

Renal failure markedly reduces renal clearance, as well as metabolic clearance of nicotine and cotinine (Molander et al., 2000). Reduction of renal clearance is correlated with the severity of kidney failure; renal clearance is reduced by half in mild renal failure, and by 94% in severe renal impairment. Markedly elevated levels of serum nicotine have been detected in smoking patients with end-stage renal disease undergoing hemodialysis (Perry et al., 1984). This is explained not only by reduced renal clearance, but also by lower metabolic clearance of nicotine in renal disease. It is speculated that accumulation of uremic toxins inhibits CYP2A6 activity or down-regulate CYP2A6 expression in liver.

B. Excretion in Feces and Sweat

In one study, the amount of radioactivity excreted in the feces of four smokers after smoking a cigarette spiked with ^{14}C-nicotine was quantitated (Armitage et al., 1975). The radioactivity detected in feces over 72 h after smoking averaged 1.0% of the estimated dose of nicotine absorbed. No radioactivity was detected in feces after intravenous administration of ^{14}C-nicotine in two subjects. Studies with rats show that a few percent of radioactivity is excreted in bile after intravenous injection of labeled nicotine, and studies with dogs and rats have detected 4 to 5% of radioactivity in feces (Schievelbein, 1982; Schepers et al., 1993). No human study has tried to quantitate the excretion of nicotine and metabolites in bile. Cotinine, but not nicotine, is detected in meconium (the first fecal matter excreted by the newborn) of children whose mothers are smokers or exposed to environmental smoke (Ostrea et al., 1994; Baranowski et al., 1998; Dempsey et al., 1999; Nuesslein et al., 1999; Derauf et al., 2003). Cotinine levels in meconium are correlated with the amount of maternal smoking (Derauf et al., 2003). Interestingly, in vitro studies with human Caco-2 cells have shown that colonic epithelial cells have an active secretion mechanism for nicotine (Fukada et al., 2002). The transporter involved in the secretion is yet to be identified.

Some nicotine and cotinine is excreted in sweat of smokers (Balabanova and Schneider, 1990; Balabanova et al., 1992; Balabanova and Krupienski, 1995; Kintz et al., 1998). Nicotine seems to be present in higher concentrations than cotinine (Kintz et al., 1998). The total amount of nicotine excreted in sweat has not been quantitated in relation to urinary excretion.

VIII. Species Differences in Nicotine Metabolism

Nicotine metabolism in various species has been reviewed previously (Gorrod and Jenner, 1975; Scheline, 1978; Seaton and Vesell, 1993). Cotinine and 3′-hydroxycotinine are major urinary nicotine metabolites in all mammalian species studied (Jenner et al., 1973; Nwosu and Crooks, 1988; Kyerematen et al., 1990a); however, about as much nicotine *N*′-oxide as cotinine and 3′-hydroxycotinine is formed by guinea pigs and rats. Guinea pig and hamster hepatocytes show the highest total metabolism of nicotine, followed by mouse, rat, and human hepatocytes (Kyerematen et al., 1990a). In general, there is considerable variation between rodent species in the activity of nicotine metabolism, as well as in the stereospecificity and relative amounts of nicotine metabolites produced. Also, P450 enzymes responsible for nicotine metabolism vary in species. For example, CYP2B1/2 is the P450 enzyme metabolizing nicotine in rats, whereas rat CYP2A is inactive in nicotine metabolism (Hammond et al., 1991; Nakayama et al., 1993).

Nicotine metabolism in nonhuman primates resembles human metabolism. In macaque monkeys, nicotine and cotinine half-lives are similar to humans (Seaton et al., 1991). Like humans, African green monkeys metabolize 80 to 90% of nicotine via a CYP2A6-like enzyme, but hepatic protein levels are about 4 times higher in green monkeys than humans resulting in 2-fold higher V_{max} for cotinine formation (Schoedel et al., 2003). Rhesus monkey hepatocytes metabolize about 80% of nicotine to cotinine (Poole and Urwin, 1976).

Nicotine *N*-glucuronidation activity is highest in human liver microsomes followed by rhesus and cynomolgus monkey microsomes, although the activity in monkey microsomes is only about 7 to 11% of human glucuronidation activity (Ghosheh and Hawes, 2002a). Low-level nicotine glucuronidation activity was also detected in minipig and guinea pig microsomes, whereas activity was not measurable in rats, mice, dogs, and rabbits. Cotinine glucuronidation was below limit of quantification for all the animal species, including rhesus, cynomolgus, and marmoset monkeys (Tsai and Gorrod, 1999; Ghosheh and Hawes, 2002a).

IX. Metabolism of Minor Alkaloids of Tobacco

Compared with nicotine, relatively little is known about the metabolism of the minor tobacco alkaloids. To our knowledge, the only published study involving administration of minor alkaloids to humans was reported by Beckett et al. (1972). This study involved determining urinary excretion of nicotine, nornicotine, anabasine, *N*-methylanabasine, β-nicotyrine, β-nornicotyrine, and myosmine following oral administration of 2-mg doses of each in two subjects. Excretion of nicotine, nornicotine, methylanabasine, and anabasine was dependent on urinary pH, with severalfold higher amounts excreted under acidic (pH 4.8) as compared with fluctuating urinary pH. With acidic urinary pH, the percentages of unchanged alkaloids recovered in urine ranged from about 15% for nicotine and methylanabasine to 70% for nornicotine and anabasine. Myosmine, β-nicotyrine, and β-nornicotyrine could not be detected in urine for the 24-h period following administration. No biotransformation products of any of the alkaloids other than nicotine were reported. In a study involving cigarette, cigar and pipe smokers, and smokeless tobacco users, the elimination half-lives of anabasine, anatabine, and nornicotine averaged 15.9, 9.6, and 11.6 h, respectively, on the basis of urine excretion rates after cessation of tobacco use (Jacob et al., 1999).

The metabolism of nornicotine in dogs following a slow intravenous infusion was reported by Wada et al. (1961). Both unchanged nornicotine and norcotinine (demethylcotinine) (Fig. 8) were recovered from urine extracts, but no quantitative data were obtained. Metabolism of nornicotine by rabbits was reported by Papadopoulos (1964). Unchanged nornicotine, norcotinine, and an unidentified metabolite were detected by thin layer chromatographic analysis following an intravenous injection or after incubation with liver homogenates.

The metabolism of nornicotine by rabbit liver microsomal preparations was studied by Nguyen (1976). Using specifically deuterated analogs of nornicotine, two isomeric pyrrolines, myosmine and 2′-(3-pyridyl)-$\Delta^{1'(5')}$-pyrroline, were identified as metabolites (Fig. 8). No norcotinine was detected in experiments with microsomal preparations, but gas chromatography-mass spectrometry analysis of extracts from incubation of nornicotine with the 10,000*g* supernatant fraction of rabbit liver did reveal the presence of norcotinine. Presumably, a soluble enzyme present in the 10,000*g* supernatant fraction is required for the conversion of 2′-(3-pyridyl)-$\Delta^{1'(5')}$-pyrroline to norcotinine. The analogous transformation of nicotine-$\Delta^{1'(5')}$-iminium ion to cotinine is catalyzed by a soluble aldehyde oxidase (Brandange and Lindblom, 1979a; Gorrod and Hibberd, 1982). Nornicotine is also converted in vitro to a nitrone, presumably via *N*′-hydroxynornicotine (Aislaitner et al., 1992).

In vitro metabolism of the minor alkaloid *N*′-methylanabasine with liver homogenates was reported by Jenner and Gorrod (1973). Unchanged methylanabasine and the diasteriomeric 1′-*N*-oxides (Fig. 8) were measured in 10,000*g* hepatic supernatant preparations from hamsters, mice, rats, and guinea pigs. Beckett and Sheikh found, in addition to the diasteriomeric *N*-oxides, products of *N*-demethylation. *N*′-Hydroxyanabasine and anabasine-$\Delta^{1'(2')}$-nitrone (Fig. 8) are formed in vitro with rat, rabbit, and guinea pig liver homogenates (Beckett and Sheikh, 1973). The same two metabolites were formed during incubations of anabasine with lung tissue homogenates. Metabolic conversion of *N*′-methylanabasine to the cotin-

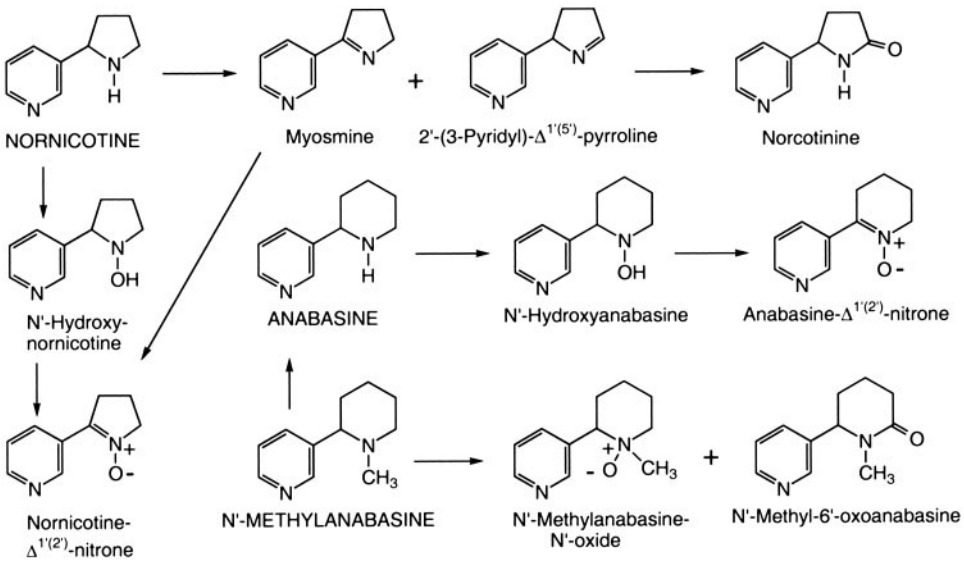

FIG. 8. Metabolism of nornicotine, anabasine, and *N′*-methylanabasine. Reprinted from Benowitz and Jacob, 1998 with permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

ine analog *N′*-methyl-6′-oxoanabasine (Fig. 8) in vitro has been demonstrated (Gorrod and Aislaitner, 1994).

Extensive metabolism of β-nicotyrine both in vitro (Shigenaga et al., 1989) and in vivo (Liu et al., 1993) has been reported (Fig. 9). In reactions catalyzed by rabbit liver and lung microsomes, β-nicotyrine is converted to a mixture of two pyrrolinones, presumably via an epoxide intermediate (Shigenaga et al., 1989). Since epoxides are electrophilic compounds capable of alkylating biomacromolecules, this pathway could have toxicological significance. It is interesting to note that nicotyrine inhibits the metabolism of nicotine in mice in vivo, resulting in higher nicotine tissue levels, without increasing its toxicity (Stalhandske and Slanina, 1982). Nicotyrine also inhibits human CYP2A6 enzyme in vitro (Denton et al., 2004). Thus, the inhibition of CYP2A6 by β-nicotyrine may explain the lower nicotine clearance detected in smokers compared with nonsmokers (Lee et al., 1987; Benowitz and Jacob, 1993, 2000). In vivo in rabbits (Liu et al., 1993, 2000), the major metabolite of β-nicotyrine was found to be *cis*-3′-hydroxycotinine along with small amounts of 5′-hydroxycotinine and 5-hydroxy-1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one (Fig. 9). The pathways leading to the transformation of β-nicotyrine to *cis*-3′-

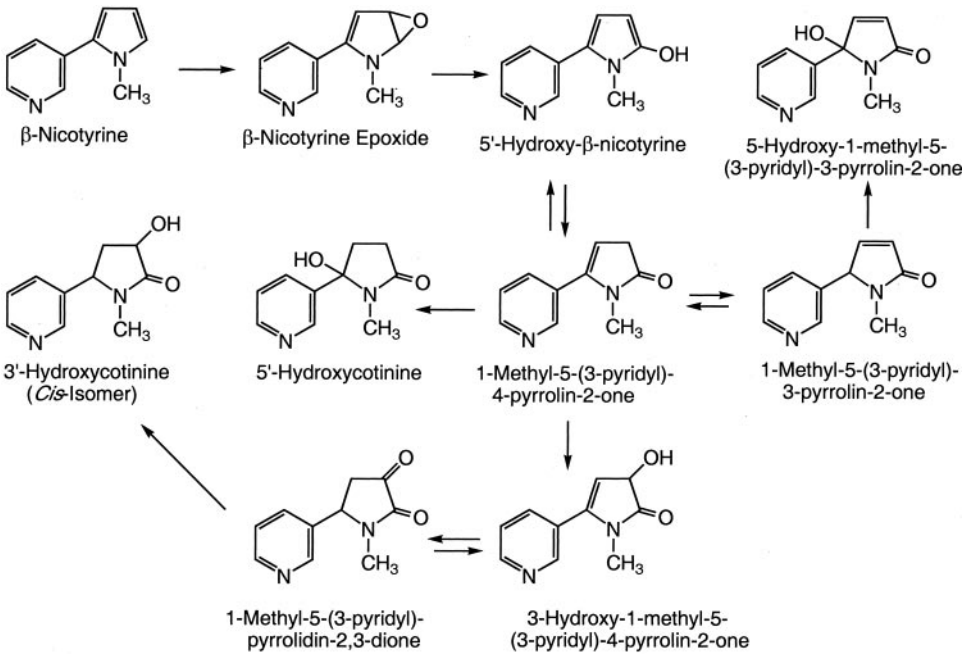

FIG. 9. Metabolism of β-nicotyrine.

hydroxycotinine and 5-hydroxy-1-methyl-5-(3-pyridyl)-3-pyrrolin-2-one were recently elucidated (Liu et al., 1999, 2000).

X. Pharmacokinetics and Metabolism of Nicotine Analogs

Numerous nicotine analogs that are of potential therapeutic utility have been described in the literature (Holladay et al., 1997; Jain, 2004). These include ABT-418, ABT-089, ABT-594, SIB-1765F, SIB-1508Y, RJR-2403, GTS-21 or DMXB, SIB-1663, A-85380, and epibatidine (Fig. 10) (Anderson et al., 1995; Bencherif et al., 1996; Cosford et al., 1996; Davila-Garcia et al., 1997; Holladay et al., 1997; Menzaghi et al., 1997; Sullivan et al., 1997; Bannon et al., 1998; Vernier et al., 1998; Khan et al., 2001). Most of these compounds have similarities in either the pyridine or pyrrolidine ring to nicotine and might be expected to undergo some of the same metabolic reactions.

The metabolism of ABT-418, an experimental drug for attention deficit hyperactivity disorder and Alzheimer's disease, has been studied in some detail, both in vitro in several animal species and in human liver slices (Fig. 11) (Rodrigues et al., 1994, 1995). Pathways of metabolism are similar to those of nicotine, including formation of a lactam (similar to cotinine), a 3′-hydroxy lactam, *cis*- and *trans*-*N*′-oxides, and *N*-desmethyl ABT-418. In human and monkey liver slices, the lactam metabolite predominates; in dog and rat liver, the *trans*-*N*′-oxide metabolite predominates. As for nicotine, the lactam formation is mediated by the P450 system, whereas *N*′-oxidation is mediated by FMO3. Cytosolic aldehyde oxidase was found to be a second enzyme involved in the metabolism of ABT-418 to its lactam, which is similar to the role of aldehyde oxidase as a second step enzyme for the conversion of nicotine to cotinine. ABT-418 has been reported to have poor oral bioavailability in animals and has been delivered to humans in clinical trials via transdermal delivery systems.

Metabolism of another nicotine analog GTS-21, a drug candidate for Alzheimer's disease, shows no particular similarity with nicotine metabolism, as it is *O*-demethylated by CYP1A2 and CYP2E1 (Azuma et al., 1999; Kitagawa et al., 2003). Little metabolism of epibatidine, a potent analgesic, is detectable in human liver microsomes, although dog and rhesus monkey microsomes show extensive stereoselective hydroxylation and *N*-oxidation (Watt et al., 2000).

XI. Conclusions and Areas for Further Study

In conclusion, our review has focused on the metabolism and disposition kinetics of nicotine, related tobacco alkaloids, and nicotine analogs. The metabolism and kinetics of nicotine and metabolites are being unraveled

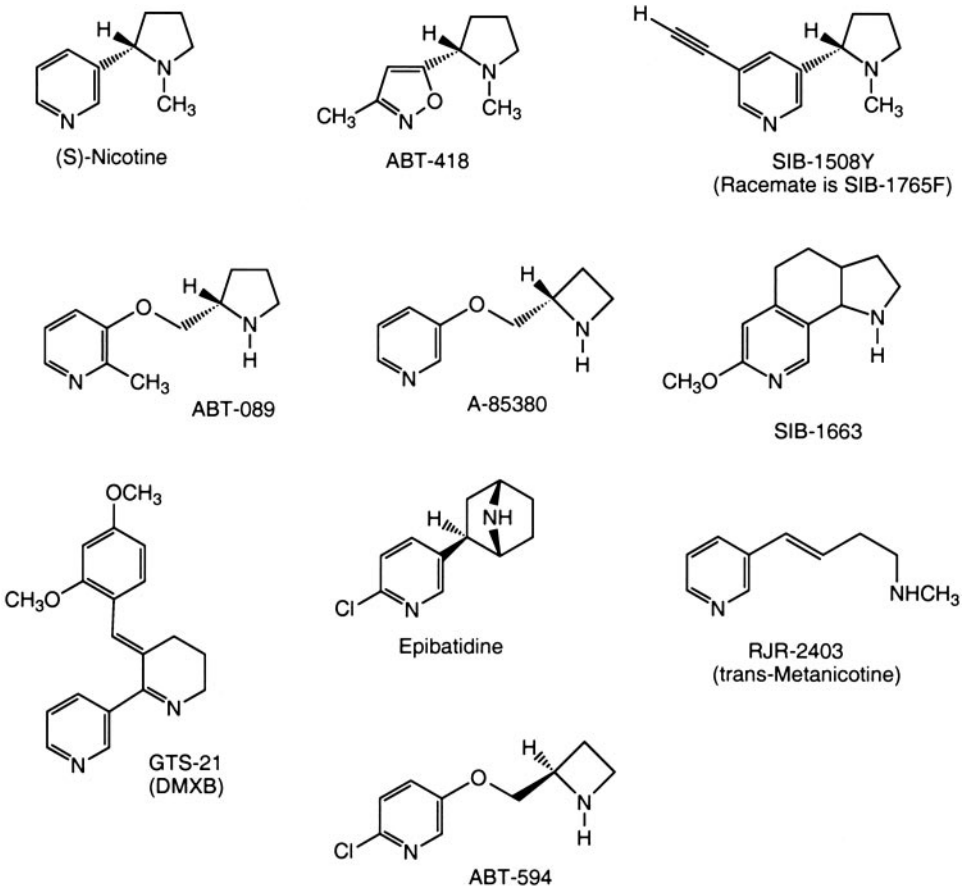

FIG. 10. Structures of nicotine analogs.

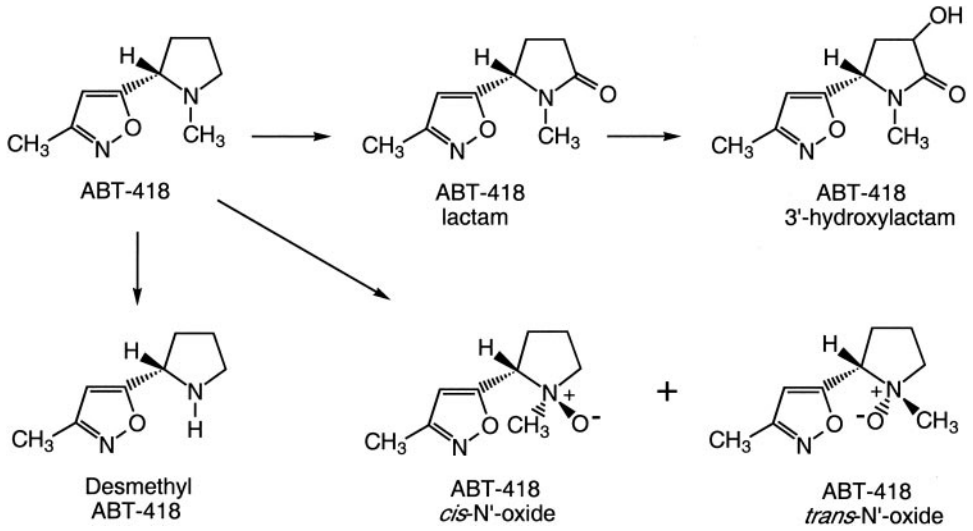

FIG. 11. ABT-418 metabolic pathways. Reprinted from Benowitz and Jacob, 1998 with permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

with increasing precision. The enzymes involved in the nicotine metabolism and factors affecting the interindividual differences, such as the genetic polymorphisms of CYP2A6 enzyme and the induction of nicotine metabolism by sex hormones, have been elucidated in recent years. New pathways of nicotine metabolism have been recently discovered. New methods to deliver nicotine as a medication have been developed and are being applied to treatment of nicotine-dependent smokers.

We have reviewed data on minor tobacco alkaloids and nicotine analogs that might be used therapeutically, but relatively little information has been published. However, because of similar structures, it is likely that many of the features of nicotine disposition will be shared by other nicotinic agonists. Pharmacokinetic considerations, including the importance of rate and route of dosing, are likely to influence the pharmacologic activity of these nicotine analogs, as they do for nicotine itself.

There are areas that need further research. Enzymes involved in some metabolic conversions of nicotine and metabolites are yet to be characterized. For example, P450 enzymes responsible for the metabolism of nicotine to nornicotine and conversion of cotinine to cotinine *N*-oxide are unknown. Furthermore, some pathways of nicotine metabolism in humans are still unresolved. Knowledge on the glucuronidation of nicotine and metabolites is less detailed than data on oxidative metabolism. The enzymes involved in the constitutive and inducible 3′-hydroxycotinine glucuronidation are unidentified. Human extrahepatic nicotine metabolism is poorly understood. Although CYP2A6 is the main enzyme influencing nicotine clearance, CYP2A13 and other nicotine metabolizing enzymes may have local importance in extrahepatic organs.

Some *CYP2A6* alleles are yet to be studied in relation to nicotine and especially cotinine metabolism. Also ultrarapid CYP2D6 metabolizers (*CYP2D6* gene amplification) should be studied in relation to nicotine and cotinine metabolism, and the role of *UGT1A4* and *UGT1A9* alleles in the polymorphic nicotine and cotinine glucuronidation in African-Americans should be elucidated.

The effect of environmental factors such as dietary compounds, smoking, cadmium exposure, and a variety of inhibitors and inducers should be studied in more detail. The mechanisms of induction of CYP2A6 enzyme and nicotine metabolism by medications such as oral contraceptives, hormone replacement therapies, and classic inducers dexamethasone, phenobarbital, and rifampicin need further study. Understanding the mechanisms of decreased hepatic nicotine metabolism by renal failure and hepatic diseases would give more insight into the regulation of nicotine metabolism. Dietary inhibitors and inducers might partly explain the high interindividual variability in nicotine metabolism. Thus, effects of menthol, grapefruit juice, wheatgrass juice, and others should be studied. The mechanism of the effect of smoking to inhibit nicotine clearance has not been explained.

Recent advances in nicotine metabolism research have elucidated some of the causes for the wide interindividual differences in metabolic capacity. Polymorphisms of the *CYP2A6* gene have a major impact on nicotine clearance. Gender-related effects, disease states, and various inhibitors and inducers affect individual rates of nicotine metabolism. However, to date, a major portion of the variability in nicotine metabolism and clearance is unexplained. Nicotine metabolism and the factors affecting it remain intriguing subjects for future studies.

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