Biocatalysts in Detoxication of Drugs of Abuse

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INTRODUCTION

Detoxication of drugs of abuse has generally followed a traditional approach involving pharmacological intervention in the biochemical action of the abused substance. This intervention has usually taken the form of the development of inhibitors or antagonists of biological macromolecules such as central nervous system (CNS) receptors. Some of the shortcomings of this approach include the fundamental biological activity of the antagonist, the possible occurrence of side effects or pharmacological liabilities of the antagonist itself, and the stoichiometric nature of the antagonism.

The idea that biocatalysts (e.g., enzymes, catalytic antibodies, ribozymes) could play a role in human therapy is not a new one. However, few examples of the application of biocatalysts to the detoxication of drugs of abuse have been reported. The use of biocatalysts in the detoxication of drugs of abuse has tremendous potential. First, the catalyst would likely have no intrinsic pharmacological activity. That is, the biomacro- or small-molecule catalyst would probably have minimal direct effect on CNS receptors. As such, the biocatalyst itself should have a markedly reduced potential for pharmacological side effects. Of course, numerous advances in "humanizing" biocatalysts must be undertaken before they are useful in the clinical setting, but it is likely that those advances will come to fruition in the near future based on the recent rapid progress of recombinant biological approaches to developing human therapeutics. Finally, biocatalysts work in an exponential catalytic fashion; it is possible that less material will be needed to be employed therapeutically, thus decreasing the possible side effects and increasing the therapeutic efficacy as a detoxication agent.

In principle, there are two fundamentally different ways to develop biocatalysts useful in detoxication of drugs of abuse. The first approach involves the procurement or design of a small molecule or macro-molecule that will selectively catalyze the detoxication reaction of interest. The second approach relies on selection of a catalyst from a large pool of candidates. However, several important

but difficult questions associated with such an exercise need to be addressed. For example, what is the critical rate-limiting step in the chemical detox-ication process? How will the catalyst be synthesized or otherwise obtained? Will the detoxication catalyst be of clinical relevance?

Selection of a catalyst for specific chemical detoxication reactions from a large biologically or chemically generated library also poses several important questions. How does one create a large biologically or chemically generated library of putative detoxication catalysts? How will the library be screened for catalytic detoxication activity? Are dependable methods available to amplify the catalyst to reasonable amounts for further study? Is it sufficient to simply screen existing stockpiles of manmade chemicals or natural products for detoxication catalytic activity, or is it imperative to develop fundamentally new technology so that novel approaches and novel agents are introduced for evaluation? It is likely that the time to develop new technology to address important questions of drug abuse is now; traditional approaches are limited, and have not appeared to solve the clinical problems thus far.

There are a number of secondary questions that arise from a consideration of the above-mentioned approaches to procuring detoxication catalysts or developing new detoxication strategies. Of course, one of the first such questions is: Will the catalysts obtained by design or selection possess any clinical relevance or be useful in human therapy? If so, will the clinical use be limited to acute toxic overdose situations or will the catalyst be useful only in drug cessation paradigms? Will the catalyst play any role in decreasing or reversing addiction liability of drugs of abuse? To a certain extent these latter questions are somewhat premature (like the field of catalyst design and selection itself). At present, a larger question should be: What is the fundamental technology useful in discovering catalysts or underlying principles of catalysis that might later support rational design? What can the technology reveal about the way detoxication catalysts (such as they are) naturally evolved? How should a drug of abuse detox-ication catalyst be evaluated in the clinic?

Several fundamentally distinct approaches to the procurement and evalu-ation of biocatalysts useful in the detoxication of drugs or chemicals of abuse are outlined below. The author has arbitrarily limited the presen-tation to a few select detoxication catalysts in the field of drug abuse. In some cases, other nondetoxicating biochemical routes of transformation are included as well.

The first section focuses on naturally occurring adult human biocatalysts that transform (*S*)-nicotine. (*S*)-Nicotine is discussed because it is one of the most well-studied CNS-active compounds and because it constitutes perhaps the most widely used pharmacologically active agent in humans. In the second section, discussion of the metabolism of cocaine focuses on cocaine hydrolysis as a major route of detoxication. Cocaine has been chosen because it illustrates some of the advantages and pitfalls of the use of cocaine esterolytic catalytic antibodies as a new potential therapeutic approach. Finally, a brief discussion of catalytic ribonucleic acid (RNA) (i.e., ribozymes) is presented. The use of catalytic RNAs as therapeutic agents is in its infancy but some of the relevant concepts discussed for enzymes or catalytic antibodies are also relevant to ribozymes. In addition, other principles observed in RNA catalysis have found considerable application in the use of oligonucleotides in antisense therapy, and there may be some useful extensions of antisense drug design that could be useful in the field of detoxication catalysts.

ENZYMES IN (S)-NICOTINE METABOLISM AND DETOXICATION

(*S*)-Nicotine[1] is one of the most widely used psychoactive drugs in the world. Almost 25 percent of adult Americans smoke despite convincing evidence of the health hazards of smoking (Surgeon General 1988). Nicotine causes complex CNS, behavioral, cardiovascular, and endocrine neuromuscular effects in humans (Benowitz 1988). Most notably, nicotine is the primary cause of tobacco addiction in humans (Surgeon General 1988). The molecular basis for tobacco addiction is unknown but it is possible that a biologically active metabolite of nicotine plays a role in nicotine tolerance and dependence, and individual differences in the metabolism and disposition of nicotine may explain why some humans become highly dependent on nicotine and others do not. Clearly, a full appreciation of the role of human enzymes in nicotine metabolism and detoxication could provide much insight into the interindividual variation in the biological responses to smoking and the variation in the pharmacological effects of nicotine.

The untoward biological properties of nicotine and nicotine metabolites are controversial. Extensive epidemiological studies have supported a role of smoking in several types of cancer. However, although nicotine is a prominent component of cigarette smoke, there are thousands of other chemicals present in tobacco smoke, including most classes of known chemical carcinogens (Hecht and

Hoffmann 1989). The determination of the agent(s) in tobacco smoke responsible for causing cancer has been and continues to be an extremely challenging problem. Most notable for this discussion, however, is that several nicotine-related metabolites are formed in humans in apparent detoxication processes. Study of these enzymatic processes could reveal why certain people are less susceptible to the harmful effects of nicotine. That is, certain humans may be endowed with the ability to decrease the potential untoward effects of nicotine by an abundance of detoxication processes. Careful study of the enzymes involved in nicotine detoxication could provide valuable clues for designing new catalysts useful in decreasing the pharmacological effects of nicotine. For example, nicotine-, cotinine-, and 3-hydroxy-cotinine glucuronides are all formed in apparent enzymatic detoxication processes. The apparent detoxication of nicotine by glucuronidation metabolic pathways in humans is quite variable and it is possible that an undiscovered relationship exists between glucuronidation and susceptibility to the untoward effects of nicotine ingestion. While nicotine addiction is a complex phenomenon involving many biological, behavioral, and other parameters, it is unknown what relationship, if any, exists between metabolic biotransformation of nicotine and nicotine addiction.

Even though the pharmacokinetics and metabolism of nicotine have been extensively studied (Beckett et al. 1971; Benowitz and Jacob 1994; Booth and Boyland 1970; Byrd et al. 1992; Caldwell et al. 1992; Jacob et al. 1988; Kyerematen et al. 1990; Neurath et al. 1987; Schepers et al. 1992; Scherer et al. 1988), the molecular basis for addiction to nicotine remains unclear. Following smoking or intravenous (IV) infusion, the terminal half-life of nicotine is about 2 hours (and is urine pH-dependent) (Benowitz et al. 1982). Total clearance averages 1300 milliliters per minute (mL/min) (and is highly variable between individuals) and the relatively large volume of distribution (183 liters (L)) explains the relatively long half-life in the presence of rapid clearance. Plasma protein binding of nicotine is very low (Duan et al. 1991). In humans, nicotine is rapidly and extensively metabolized. Liver monooxygenases and, to a lesser extent, lung monooxygenases have been implicated, and at present about 90 percent of ingested nicotine can be accounted for as urinary metabolites (Benowitz and Jacob 1994). The major excreted urinary metabolites of nicotine in humans are cotinine (10 to 15 percent),

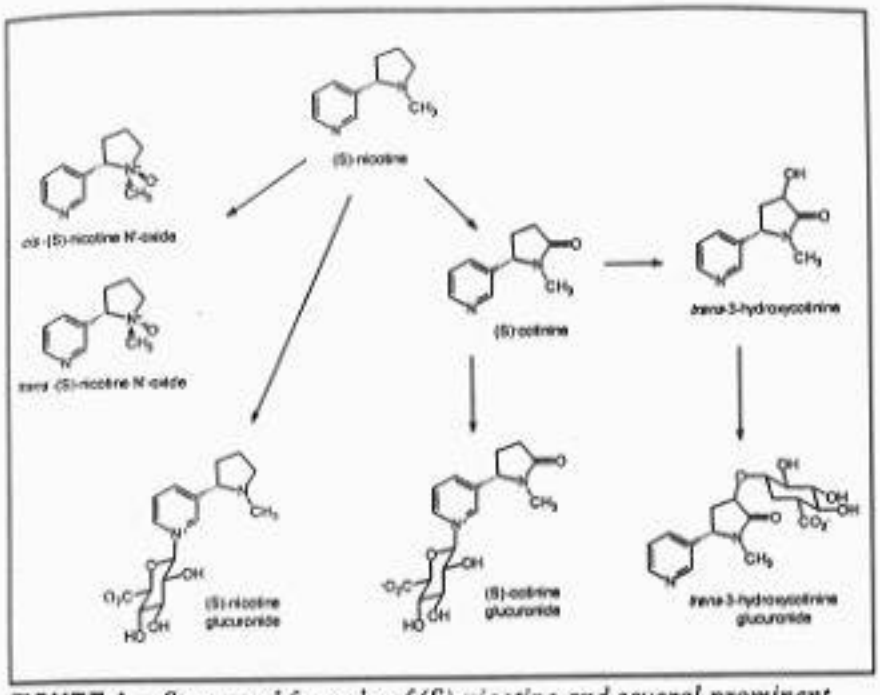

FIGURE 1. *Structural formula of (S)-nicotine and several prominent metabolites.*

nicotine N-1'-oxide (4 percent), *trans*-3-hydroxycotinine (30 to 35 percent) and cotinine-, 3-hydroxycotinine-, and nicotine-glucuronides (together, approximately 24 percent) (figure 1) (Benowitz and Jacob 1994). Cotinine and nicotine N-1'-oxide have been used as markers of nicotine exposure. Cotinine has a long half-life (16 hr) and blood levels are stable (Benowitz et al. 1983). However, it may be the glucuronide metabolites of nicotine or cotinine that are the most useful as bioindicators of nicotine exposure. Regardless, cotinine and nicotine N-1'-oxide have historically been used to verify human nicotine exposure.

In 26 smokers, mean cotinine excretion was 1.39 mg/24 hr, while nicotine N-1'-oxide was excreted at a rate of 0.56 mg/24 hr in the same smokers (Jacob et al. 1986). The disposition of nicotine N-1'-oxide in humans has been characterized (Park et al. 1993). Administration of deuterated nicotine N-1'-oxide (figure 2) to humans results in the rapid excretion of the N-1'-oxide largely unchanged in the urine. Examination of the stereochemistry of the nicotine N-1'-oxide before and after administration showed that the stereochemistry of the excreted material was essentially identical to that of the administered compound. That no

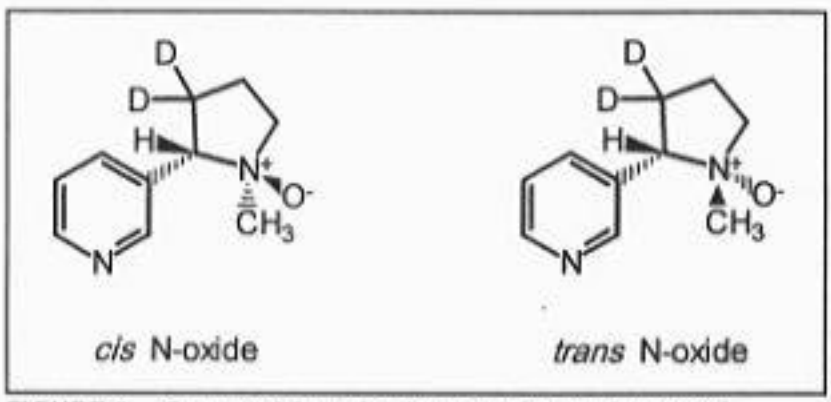

FIGURE 2. *Structural formulas for cis and trans deuterated (S)-nicotine N-1'-oxide.*

change in stereochemistry and no reduction of IV-administered N-1'-oxide to nicotine was observed in humans (Beckett et al. 1971; Park et al. 1993) suggests that N-1'-oxygenation of nicotine is a detoxication process. In animals, considerable reduction of oral or intraperitoneally (IP)-administered nicotine N-1'-oxide was observed (Dajani et al. 1975). In rabbits, less than 3 percent is reduced following IV dosing, but after oral administration 45 percent of nicotine N-1'-oxide is reduced. It is likely that gut bacteria or intestinal reductases are responsible for the reduction of nicotine N-1'-oxide after oral administration (Duan et al. 1991). In rabbits, nicotine N-1'-oxide clearance was 7.5 mL/min per kilogram (kg), had a half-life of 42.6 min, a volume of distribution of 340 mL/kg, and was not plasma protein bound (Duan et al. 1991). The idea that nicotine N-1'-oxide serves as a reservoir for nicotine (i.e., by reduction back to nicotine) and participates in maintaining nicotine levels required for addiction and nicotine-related toxicity is apparently not true in humans (Park et al. 1993). This is not to imply that nicotine N-1'-oxide does not have pharmacological activity; it causes vasoconstrictive effects on isolated rabbit-ear vessels and increases the tonus of isolated rabbit intestine, and in mice causes qualitatively similar tremor, dyspnea, and spasms that are similar (IV administration) in effects to nicotine, but the dose required is much larger (Barass et al. 1969). More important, the N-1'-oxide administered before challenge with nicotine conferred protection against a subsequent lethal dose of nicotine (Barass et al. 1969). The conclusion is that formation of nicotine N-1'-oxide constitutes a true detoxication route of disposition, but in most adult humans, this pathway represents only a small percentage of the overall metabolism of nicotine. In principle, development of catalysts that could form

nicotine N-1'-oxide may be a useful adjuvant in smoking cessation therapy.

Cytochromes P-450

Human cytochrome P-450 is a ubiquitous heme-containing enzyme that participates in the oxidation of a wide variety of chemicals, drugs, and even some endogenous materials (Guengerich and Shimada 1991). Although the cytochrome P-450 transformation of nicotine has been extensively described for animals, nicotine has not been extensively used as a probe substrate for adult human metabolism. The molecular basis for metabolism of nicotine in humans is considerably different from that observed in animals (Cashman et al. 1992). In humans, nicotine is primarily eliminated by metabolism, with total clearance varying by about fourfold among individuals. Men metabolize nicotine more rapidly than women (Benowitz and Jacob 1984). Age also influences urinary nicotine metabolite levels in humans. The pathway leading to cotinine is the major route of nicotine metabolism, and the initial metabolic step is nicotine $^{1',5'}$-iminium ion formation. In previous studies, the author and colleagues identified human liver cytochrome P-450 2A6 as the principal enzyme responsible for nicotine $^{1',5'}$-iminium ion formation (Cashman et al. 1992). This result is in contrast to the situation in animals where cytochrome P-450 2B (identical to the phenobarbital-inducible form) has most often been implicated as the putative nicotine oxidase (McCoy et al. 1989; Williams et al. 1990). In rats and rabbits, phenobarbital pretreatment increased cotinine formation in liver microsomes supplemented with aldehyde oxidase that was isolated from the animals. In the absence of aldehyde oxidase, cytochrome P-450 is responsible for the metabolism-dependent covalent binding of nicotine to tissue macromolecules, implicating nicotine $^{1',5'}$-iminium ion as an electrophilic metabolite (Williams et al. 1990; Shigenaga et al. 1988). In rabbit lung microsomes, cytochrome P-450 2 (or LM2), which is similar to the phenobarbital-inducible hepatic form, is primarily responsible for nicotine $^{1',5'}$-iminium ion formation that can be trapped by cyanide or converted to cotinine by aldehyde oxidase (Williams et al. 1990). It is important to note that the cytochrome P-450 corresponding to the rat or rabbit liver phenobarbital-inducible form (i.e., adult human liver cytochrome P-450 2B6) is highly variable and is detectable in less than 3 percent of adult human livers examined (Mimura et al. 1993).

Because there is strong evidence that P-450 2A6 is the primary enzyme forming nicotine $^{1',5'}$-iminium ion in adult human liver

microsomes, it is likely that in human tissues about 90 percent of the nicotine iminium ion formation is mediated by cytochrome P-450 2A6 (Berkman et al. 1995; Cashman et al. 1992). Interestingly, increased hepatic cytochrome P-450 2A6 levels have been observed in humans with a history of barbiturate administration (Cashman et al. 1992). Production of nicotine $^{1',5'}$-iminium ion showed some dependence on the previous drug administration history of the subject from which the hepatic microsomes were isolated (table 1). Two of the highest rates of iminium ion formation occurred in the presence of human liver microsomes from barbiturate-pretreated subjects (i.e., E and I) (table 1). Of the seven major human cytochrome P-450 enzymes examined (i.e., 1A2, 2A1, 2C8, 2C9, 2D6, 2E1, 3A5, and 3A total), the greatest linkage between immunoreactivity and nicotine iminium ion formation (i.e., at 10 micromolar (M) substrate concentration) was observed for cytochrome P-450 2A6 (r = 0.9) (Berkman et al. 1995). Neither gender, age, nor smoking history provided a direct relation between nicotine $^{1',5'}$-iminium formation and cytochrome P-450 2A6 levels. Others have suggested that cytochrome P-450 2D6 or 2B6 plays a role in adult human liver nicotine oxidase activity. However, these studies employed extremely high nonphysiological concentrations of nicotine (Flammang et al. 1992). Based on their studies, the author and colleagues anticipate that non-cytochrome P-450 2A6 enzymes contribute less than 10 percent to the oxidation of nicotine (Berkman et al. 1995).

In summary, in adult human liver, cytochrome P-450 2A6 is responsible for formation of nicotine $^{1',5'}$-iminium ion that is subsequently converted by aldehyde oxidase to cotinine. Because cotinine is extensively glucuronidated or metabolized to 3-hydroxy-cotinine (which is in turn glucuronidated), detoxication of nicotine via the cotinine glucuronidation pathway must pass through the relatively electrophilic iminium ion pathway. However, with the exception of a few studies (most notably by Castagnoli and coworkers) (Peterson and Castagnoli 1988; Peterson et al. 1987), the pharmacological or toxicological effects of nicotine $^{1',5'}$-iminium ion have not been reported. It is interesting to note that human liver cytochrome P-450 2A6 and 2E1 catalyze metabolic activation of the tobacco smoke-related nitrosoamines 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), and N-nitrosonornicotine (NNN). Thus, cytochrome P-450 2A6 and 2E1 could catalyze the conversion of several tobacco smoke-related nitrosoamines to genotoxic products (Yamazaki et al. 1992).

				Metabolite formed [pmol/(min mg of protein)]				
patient code	gender	age	drug history	FMO immuno-reactivity [b]	nicotine N-1[1]-oxide	cotinine	nornicotine	
A	M	25	none	3	226	294	ND	
B	M	50	none	2	88	238	ND	
C	M	22	ethanol	2	181	187	ND	
D	M	31	MPME	1	54	568	ND	
E	M	14	pentobarb	2	180	376	ND	
F	F	50	ethanol	2	55	353	ND	
G	F	48	teldrin	5	225	299	ND	
H	F	28	none	2	34	284	ND	
I	N	43	phenobarb	3	45	750	ND	
J	F	55	none	4	133	377	ND	
K	M	23	ethanol	4	192	431	ND	
L	F	58	dopamine	4	141	208	ND	
M	M	18	ethanol	3	67	234	ND	
N	M	21	ethanol	2	127	311	ND	

KEY: a = Incubations were performed with 0.4-2.0 mg of microsomal protein, 4.3 mg of rat liver microsome supernatant (as a source of aldehyde oxidase), 0.5 mM NADPH at pH 8.4 and 37%C for 10-20 min, and products were quantified by HPLC. For cotinine, nornicotine, and 7-OH-coumarin formation, incubations were at pH 7.4. Values are the mean of 2-4 determinations 10%. b = Relative immunoquantitation; 5 = greatest, 1 = weakest, ND = not detected.

SOURCE: From Cashman et al. 1992, used with permission.

The conclusion of the study was that interindividual variations in the amount of human cytochrome 2A6 and 2E1 could contribute to the susceptibility of environmental procarcinogens including those derived from tobacco smoke. Cytochromes P-450 largely bioactivate nicotine and nicotine-related nitrosoamines to electrophilic materials. Presumably, the balance between bioactivating enzyme activity and detoxicating enzyme activity is important in determining the overall susceptibilities of humans to exposure to chemicals. Below, the properties of several additional enzymes in nicotine detoxication are described.

Aldehyde Oxidase

Aldehyde oxidase is a cytosolic, iron-containing molybdoflavoprotein that mediates the oxidation of aldehydes and nitrogen-containing heterocyclic drugs, chemicals, and endogenous substrates (Beedham 1985). The structural biology of the various forms of the enzyme are just beginning to be described, but animal and human aldehyde oxidase apparently consists of homogenous homodimers of subunit molecular size (approximately 150,000 daltons (Da)). The substrate specificity of human liver aldehyde oxidase has not been as exhaustively examined as animal liver aldehyde oxidase but some data point to a similar spectrum of substrate activity (Beedham 1985; Krenitsky et al. 1974). Thus, prototypic substrates such benzaldehyde, 6-methylpurine, and N-1-methylnicotinamide appear to be good substrates for both animal and human forms of aldehyde oxidase (Rodrigues 1994).

Electronic factors and the relative lipophilicity of the molecule probably help to determine the affinity of the substrate for the enzyme as well as turnover properties. It is likely that the fundamental instability of the enzyme has hampered progress in the characterization of human liver aldehyde oxidase. At least in animals, the specific activity of the enzyme is quite dependent on the way the tissue is procured, processed, and stored; this may lead to considerable intersample variability. Enzyme instability may at least in part explain why aldehyde oxidase activity from different species is so variable (Duley et al. 1985). However, it is likely that in addition to intrinsic differences in stability, the determination of aldehyde oxidase activity for a given substrate in various tissue preparations is dependent on the analytical methodology employed to assay the enzyme and the likelihood of the presence of different forms of the enzyme that possess distinct substrate specificity and kinetic properties (Johns 1967; Beedham 1985). For example, in the

presence of six different adult human liver preparations (i.e., the 9000 *g* supernatant or S-9 fraction), the oxidation of benzaldehyde, 6-methylpurine, and N-1-methylnicotinamide by aldehyde oxidase varied by 3.6-, 2.3-, and > 40-fold, respectively (Rodrigues 1994) (table 2).

TABLE 2. *Aldehyde oxidase activity in human liver 9000 g supernatant (S-9) fractions.*

				Activity* (nmol/ min/mg S-9 protein	
Subject code	Age (years)	Sex	BA	6-MP	NMN
FGL	26	F	11.4	17.5	1.4
GDD	25	F	14.5	14.7	<0.5
GEQ	20	M	19.5	14.1	<0.5
GFE	50	F	40.7	32.8	20.4
GC4	29	M	26.3	23.1	<0.5
FRX	58	M	22.2	15.5	1.5
Mean Å SD			22.4 Å 10.4	19.6 Å 7.2	7.78 Å 10.9
Fold variation**			3.6	2.3	>40

KEY: * = Data for each subject represent the mean of duplicate determinations. BA = benzaldehyde; 6-MP = 6-methylpurine; NMN = N-1'-methylnicotinamide. ** = Ratio of the highest/lowest activity.

SOURCE: From Rodrigues 1994, used with permission.

In agreement with what has been observed in animals, the level of aldehyde oxidase activity present in human liver is also markedly dependent on the substrate employed, the analytical method used to evaluate the substrate, and most importantly, the intrinsic activity of the preparation (Beedham et al. 1992). For N-1-methylnicotinamide oxidase activity, the rank order was cynomolgus monkey > rat > beagle dog > human liver (Rodrigues 1994). The relative levels of aldehyde oxidase activity may help determine the relative extent of lactim formation from N-heterocyclic compounds such as N-1-methylnicotinamide. Of course, formation of lactim metabolites from iminium ions can also be coordinately regulated by other enzyme activities including cytochromes P-450. Thus, formation of

iminium ions by cytochrome P-450 and conversion to lactams by aldehyde oxidase can be thought of as an important detoxication mechanism whereby N-heterocyclic compounds are converted into more polar materials that are excreted (Ohkubo and Fujimura 1982). In addition to its role as an oxidative enzyme, aldehyde oxidase has been reported to reduce a number of tertiary amine N-oxides back to the parent tertiary amine (Kitamura and Tatsumi 1984*a*, 1984*b*). Because a number of heterocyclic tertiary amines can undergo sequential 1-electron oxidation (i.e., via the iminium ion by cytochrome P-450) or 2-electron oxygenation (i.e., to the tertiary amine N-oxide by the flavin-containing monooxygenase), the participation of aldehyde oxidase activity in the disposition of tertiary amines may represent an important aspect of drug of abuse detoxication.

Flavin-Containing Monooxygenase

The mammalian flavin-containing monooxygenase (FMO) is a widely distributed membrane-associated family of enzymes that catalyze the oxygenation of nucleophilic nitrogen-, sulfur-, and phosphorous-containing xenobiotics, drugs, and endogenous substances (Ziegler 1993). Nucleophilic tertiary amines such as nicotine are readily converted to relatively stable tertiary amine N-oxides that are more polar and readily excreted unchanged (Park et al. 1993). As described above, nicotine N-1'-oxygenation is a route of detoxication for nicotine in humans. In contrast to cytochromes P-450, the mechanism of human FMO form 3 (FMO3)-catalyzed N-1'-oxygenation of nicotine involves 2-electron oxygenation to provide exclusively the trans nicotine N-1'-oxide (Cashman et al. 1992). Formation of nicotine N-1'-oxide diastereomers is highly FMO enzyme form-dependent. Thus, pig FMO1 forms approximately a 40:60 mixture of cistrans nicotine N-1'-oxides (Damani et al. 1988; Park et al. 1993). In contrast, human stereoselective formation of trans nicotine N-1'-oxide may serve as a highly sensitive stereochemical probe of adult human FMO3 activity in vitro and in vivo.

In the presence of liver microsomes, human FMO3 nicotine N-1'-oxygenase activity is not strongly dependent on the age, gender, or drug administration history of the subject from whom the liver was obtained (Cashman et al. 1992). In good agreement with in vitro studies, in vivo metabolism of nicotine in humans by three routes of administration including ad libitum smoking, intravenous infusion of nicotine-d_2, and dermal patch administration of nicotine all produced

only the trans diastereomer of nicotine N-1'-oxide in the urine (Park et al. 1993). In addition, trans nicotine N-1'-oxide is not appreciably reduced or oxidized further because infusion of nicotine-d_2 N-1'-oxide in humans with a known ratio of cis to trans N-1'- oxide diastereomers gave recovered urinary metabolite N-1'-oxides in high yield and with metabolite diastereoisomer ratios essentially identical to that of the infusate. In addition, human administration of highly purified preparations of stereochemically characterized nicotine-N-1'-oxide resulted in the material being rapidly excreted unchanged. This data supports the idea that formation of nicotine N-1'-oxide is a detoxication process.

Although a slight difference between the average amount of nicotine-N-1'- oxide formed and excreted in the urine of smokers versus the transdermal route of administration (i.e., 3.7 versus 2.7 percent of total urinary metabolites) could possibly reflect a minor contribution from pulmonary human FMO2 (Benowitz and Peyton 1994), the preferential N-1'-oxygen-ation of nicotine by hepatic FMO as opposed to lung FMO observed in animals (Williams et al. 1990) suggests that human liver FMO3 is the major contributing metabolic pathway. Formation of trans nicotine N-1'-oxide has been proposed as a selective functional marker for adult human FMO3 activity. The fact that nicotine N-1'-oxide formation possesses significantly less pharmacological activity than nicotine suggests that catalysts designed to convert nicotine to nicotine N-1'-oxide might be useful in smoking cessation treatment.

As stated above, it is likely that human FMO3-catalyzed formation of trans nicotine N-1'-oxide constitutes a detoxication process whereby the pharmacologically active nicotine alkaloid is converted to a polar, readily excreted tertiary amine N-1'-oxide. Previously, it has been suggested that the role of FMO in human metabolism is to metabolize xenobiotics from plant sources to benign materials that do not pose a pharmacological or toxicological challenge to the organism ingesting the chemical (Ziegler 1993). Because many CNS drugs commonly abused by humans contain a tertiary amine center, elaboration of catalysts to form tertiary amine N-oxides could constitute the basis for a new class of detoxication catalysts.

URIDINE DIPHOSPHOSPHATE GLUCURONOSYL TRANSFERASE

Glucuronosyl transferases mediate the transfer of uridine-5'-diphospho-- D-glucuronic acid to acceptor molecules in metabolic transformations that are generally considered detoxication reactions. While acyl glucu-ronides in some cases have been associated with potentially toxic conse-quences, in most instances, formation of glucuronide metabolites is associated with detoxication reactions. In humans, there have been numerous reports of the urinary excretion of quaternary N-linked glucu-ronides (Chaudhary et al. 1988; Lehman et al. 1983; Macrae et al. 1990). Recently, studies have confirmed that major metabolites of nicotine in humans are in fact glucuronide conjugates (Caldwell et al. 1992). Thus, humans administered nicotine by smoking or by a dermal patch route of administration excrete the glucuronide conjugates of (*S*)-nicotine, cotinine, and 3-hydroxy cotinine (i.e., on average, 30 percent, 49 percent, and 16 percent of a dose is conjugated, respectively) (Benowitz and Jacob 1994). While there is significant interindividual variability in the excretion of glucuronide conjugates, the pattern of metabolism is generally similar when nicotine is inhaled or absorbed transdermally. However, formation of nicotine and cotinine glucuronides does not exactly parallel the formation of 3-hydroxy cotinine glucuronide. Apparently, conjugation of 3-hydroxy cotinine is done by a different glucuronosyl transferase than the enzyme responsible for nicotine and cotinine glucuronidation (Benowitz and Jacob 1994). This may reflect the fact that one enzyme form is a nicotine N-glucuronosyltransferase while the other enzyme form is a nicotine O-glucuronosyltransferase.

Conjugation is a quantitatively important route of nicotine detoxication in humans. The large amount of nicotine glucuronosyl conjugates observed in human urine following nicotine administration and the significant degree of interindividual variability in the amount of glucuronide formed have confounded accurate assessment of nicotine metabolic disposition. By taking into account the glucuronidation pathway, a more complete quantification of nicotine metabolism and disposition could enhance the accuracy of bioindicators of nicotine exposure. In the gut, glucuronosyl conjugates of nicotine may become substrates for microflora - glucuro- nidase. As such, it is possible that enterohepatic recirculation of nicotine could arise via this pathway. A cycle of conjugation and hydrolysis could represent a mechanism to prolong the bioavailability of nicotine or its metabolites. While glucuronidation of nicotine or one of its metabolites represents a detoxication reaction, considering

other possible metabolic pathways makes the analysis of the contribution of conjugation to detoxication less straightforward.

GLUTATHIONE TRANSFERASE

While no published studies of the conjugation of electrophilic nicotine metabolites with glutathione have been reported, in principle, enzyme-catalyzed glutathione addition could produce metabolites that are more polar and more readily excreted. Nucleophilic addition of low molecular weight thiols to nicotine $^{1',5'}$-iminium ions has been shown to occur under pseudo first-order reaction conditions (Brandage and Lindblom 1979). In the liver where glutathione reaches millimolar concentrations, it is possible that glutathione conjugation contributes to the disposition of the nicotine iminium ion, especially in animals with low amounts of aldehyde oxidase. Presumably glutathione conjugates, if formed, could also be transformed into mercapturates that should be readily excreted. While investigations of a role for glutathione conjugates or mercapturates of nicotine have not been reported in the literature, the feasibility of trapping electrophilic iminium ion metabolites of nicotine with thiol nucleophiles has ample precedence (Brandage and Lindblom 1979). At this point it is only speculation that addition of biologically relevant sulfur-containing nucleophiles constitutes a detoxication reaction for metabolites of nicotine. Finally, because nucleophilic addition to the nicotine iminium ion is reversible, from a practical standpoint, an addition compound will probably only be formed and detected under conditions where the nucleophile is present in large excess.

Tobacco Addiction

Tobacco addiction is complex, and involves behavioral as well as pharmacologic factors. The importance of nicotine in tobacco dependence has been demonstrated in many studies (Benowitz and Jacob 1990; Surgeon General 1988). However, the neurochemical mechanisms of nicotine tolerance and dependence are not known, and the possibility exists that a reactive metabolite may be involved. Thus, identification of nicotine-derived electrophilic metabolites that covalently modify human proteins may provide insight into the fundamental mechanisms underlying tobacco addiction. This is a largely unexplored area of research. Knowledge of the bioactivation as well as detoxication steps in the metabolism of nicotine may help

identify the reasons for the interindividual variation and susceptibility to the injurious effects of this widely used chemical.

Cocaine Use and Related Toxicity in the United States

During recent years, cocaine has been most commonly ingested in the United States by smoking cocaine base (in chunk or crack form) (NIDA 1990). Smoking crack cocaine is preferred by abusers because the concentration of cocaine in venous blood peaks sooner after smoking crack (or after IV administration of cocaine) than by other routes of administration. Cocaine is generally not abused by the oral route of administration, but oral absorption is efficient and is a significant route of dosing as a cause of toxicity (Jones 1990). Thus, cocaine toxicity could be greater for children who have swallowed cocaine in mother's milk, for example.

Cocaine is hydrolyzed by esterases of the blood, liver, and other organs (Benowitz 1992). The major (inactive) metabolite of cocaine is benzoylecgonine, but ecgonine and ecgonine methyl ester are also formed (Benowitz 1992) (figure 3). Hepatic metabolism of cocaine to norcocaine (pharmacologically active) accounts for only 2 to 6 percent of the total amount eliminated (Benowitz 1992). After IV administration, cocaine has a plasma half-life of 70 mins. The transient nature of the CNS effects of cocaine is due to the relatively rapid redistribution of cocaine into and out of the brain and into other tissues. This type of rapid influx-efflux brain distribution is typical of lipophilic agents such as cocaine. In contrast to IV or smoking routes of administration, systemic effects and plasma levels may be sustained for longer periods because of continuous absorption after oral administration and selective binding to CNS monoamine reuptake transporters.

The primary effects of cocaine in humans are CNS stimulation resulting in euphoria and activation of the peripheral sympathetic nervous system with tachycardia and blood pressure elevation. Tolerance to the euphoric effects of cocaine develops quickly (Foltin and Fischman 1991), but is incomplete for the cardiovascular effects of cocaine (Ambre et al. 1988). It is possible that repeated use of cocaine to seek the high may lead to progressive cardiovascular toxicity. Medical problems arising from cocaine abuse are among the most common causes for emergency room visits in U.S. hospitals, especially in the inner cities (Brody et al. 1990). Three types of life-threatening medical toxicities reported include

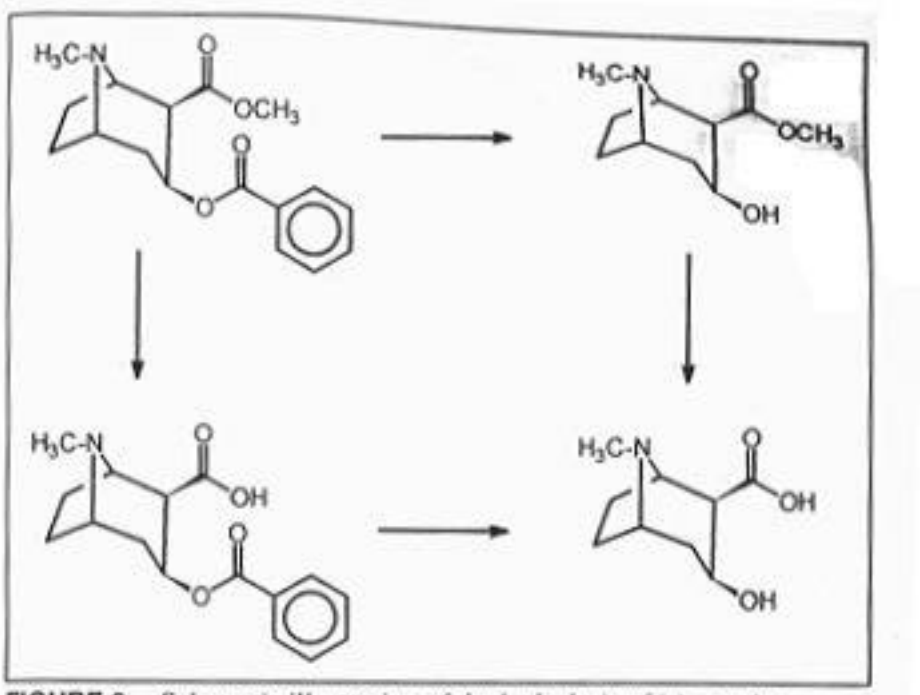

FIGURE 3. *Schematic illustration of the hydrolysis of (-)-cocaine.*

cocaine-related sudden death, cardiovascular disease, and reproductive disturbances (Benowitz 1992). Sudden death is believed to result from cardiac arrhythmias due to massive catecholamine release with or without acute myocardial ischemia. However, a review of 935 cocaine-related deaths reported in 1986 in New York City showed that the top causes of death were homicide (37.5 percent), acute narcotic exposure (12 percent), other natural causes (11.1 percent), and suicide (6.6 percent) (Litovitz et al. 1991). Another serious type of injury from cocaine use with consequences for long-term harm involves brain damage. Episodes of angina and myocardial infarction due to cocaine abuse have also become commonplace in reports from major U.S. metropolitan medical centers.

In inner-city areas of the United States, surveys of pregnant women reported rates of maternal cocaine use as high as 30 percent. Maternal cocaine use can affect reproduction by adversely affecting the pregnancy. In addition, cocaine use may directly injure the fetus and/or produce behavioral and developmental abnormalities in the neonate. Indirect toxicities of cocaine abuse may result in severe health consequences including child neglect and abuse, loss of family structure, an increased risk of AIDS, and congenitally acquired sexually transmitted diseases (Benowitz 1992). A study of data from 72 U.S. Poison Control Centers in 1990 that analyzed 1.7 million

human drug exposure cases (Litovitz et al. 1991) showed that cocaine was implicated in more major medical complications and deaths than any other chemical agent. Cocaine use among polydrug abusers, presumably occurring at increasingly higher doses via IV injection or by smoking, has become more toxic to humans. In summary, the current picture of cocaine abuse is associated with more emergency room visits and more deaths than any other illicit drug of abuse.

Completely new strategies for preventing the toxicity and abuse of cocaine are needed and novel methods may be best developed by employing modern biological and biochemical approaches (Jones 1992). It is unlikely that antagonists of neurotransmitter transporters or other pharmacological intervention (i.e., desipramine or other antidepressants) will be efficacious because the blood levels required to saturate the cocaine receptor will be too toxic in humans (Ritz et al. 1987) or have an induction period of several weeks (Fischman et al. 1990). The reasons for cocaine deaths probably stem partly from a contribution of the intrinsic toxicity and mechanism of action of cocaine and partly from the current lack of appropriate treatment for cocaine overdose. With heroin, the opiate receptor antagonist naloxone is likely to save someone brought to the emergency room with a heroin overdose. A comparable antagonist, such as the opiate antagonist naloxone, is currently not available to treat cocaine overdose. Antibodies against opiates have been reported to antagonize the reinforcing effect of low doses of heroin (Bonese et al. 1974) but because the antibodies were removed from circulation presumably by clearance processes, the therapeutic paradigm failed at high doses of heroin. Only symptomatic treatment of cocaine overdose is available today.

Powerful selective antidotes or antagonists to treat cocaine abuse are necessary to offset the increased cocaine toxicity currently observed in the United States. The use of catalytic antibodies in the creation of selective binding agents and detoxication catalysts of cocaine could represent a novel approach that may result in significant advances in the field of detoxication of drugs of abuse. An anti-cocaine catalytic antibody directed to hydrolyze the benzoyl ester could in principle catalyze the formation of ecgonine and benzoic acid, hydrolysis products of cocaine that do not possess the reinforcing or CNS stimulation properties of cocaine (Spealman et al. 1989).

Catalytic Antibodies

Antibodies are proteins that can recognize a wide array of chemicals, drugs, and other biological materials with great specificity and efficacy

(Harlow and Lane 1988). An antibody may bind to its ligand with an equilibrium dissociation constant (Kd) equal to 10^{-12} molar (M). In contrast, an enzyme typically devotes only a fraction of its overall free energy to binding phenomena and generally reserves most of its expendable free energy on catalytic events. In principle, an antibody can afford to bind a ligand with less avidity and channel the difference in free energy into catalytic activities. The fundamental similarity between antibodies and enzymes has important implications for combining the catalytic action of enzymes with the selective recognition function of antibodies.

The general approach to producing catalytic antibodies is based on the principle of reaction intermediate-enzyme transition state complementarity (Pauling 1946; Wolfenden and Kati 1991). Small molecule analogs of putative enzyme reaction transition states have been used as haptens to induce antibodies with complementary binding sites (Janda et al. 1989; Lerner et al. 1991; Pollock et al. 1986; Tramontano et al. 1986*a*, 1986*b*). As in the case of enzymes that have evolved to preferentially stabilize the transition state, antibodies induced to recognize the transition state have been shown to stabilize the putative transition state of the reaction and afford catalysis (Lerner et al. 1991; Tramontano et al. 1986*a,* 1986*b*). One of the first examples of catalytic antibody activity led to the development of hydrolytic or acyl transfer catalytic antibodies (Janda et al. 1988, 1989). Thus, simple tetrahedral carbon mimics representing the transition state for ester hydrolysis (i.e., phosphonates) were synthesized and antibodies directed against these materials (i.e., antibodies directed against small molecules covalently attached to a large carrier molecule such as a protein) possessed esterolytic activity against the corresponding ester substrate.

In principle, catalytic antibodies can be developed to facilitate selective chemical reactions that do not have biological counterparts (Benkovic et al. 1988). For example, toxins could be selectively detoxicated, novel chemical reactions could be developed, or extremely sensitive bioprobes of antibody ligands could be readily developed.

Esterolytic Catalytic Antibodies

Simple structures such as phenyl phosphonates have been synthesized and used to generate monoclonal antihapten antibodies (Tramontano et al. 1988). Antibody-producing hybridoma cells can be cloned to obtain colonies, each producing a single type of antibody

(Tramontano and Schloeder 1989). Because a large number of hybridomas can be generated, it was thought to be impractical to screen all cultures for catalytic activity. Today, with advances in rapid screening techniques, it is now practical to screen hundreds if not thousands of hybridomas for antibodies with catalytic activity. Typically, an antibody is produced in microgram quantities in cell culture and a subset of the population that recognizes the original hapten itself is chosen. One of the first catalytic antibodies studied (i.e., esterase 50D8) (Tramontano et at. 1986*a*, 1986*b*) has been well characterized. Esterase 50D8 shows a high degree of substrate specificity; the rate advantage of 6×10^6 over the background (i.e., uncatalyzed rate) and the catalytic efficiency (i.e., k_{cat} 29 sec^{-1}) both illustrate the catalytic properties of the antibodies; and the antigen or hapten inhibits the substrate (i.e., K_i=60-80 nM). However, the rate factor (i.e., k_{cat}/K_m of 10^4 M^{-1} sec^{-1}) is still several orders of magnitude less than those of a good enzyme that catalyzes an analogous reaction (i.e., values of 10^7 or 10^8 M^{-1} sec^{-1}) and the activity increases sharply above neutral pH and becomes pH-independent above pH 10. This latter observation has been found with most esterolytic catalytic antibodies, and probably reflects the fact that deacylation of the antibody by hydroxide ion is rate limiting. The design of future esterolytic catalytic antibodies must address this apparent shortcoming.

Cocaine Catalytic Antibodies

Typical antibody-catalyzed reaction rates are several hundredfold to 100,000-fold faster than the uncatalyzed reaction of the substrate. Several fundamental postulates have been proposed to explain the rate enhancements that nevertheless fall short of the enormous rate accelerations of enzymes. Is activity truly due solely to transition state stabilization by antibody-binding interactions? Can additional binding interactions be built into the combining site or into the substrate molecule itself to increase the overall rate of the reaction? Can new screening methods and immunological methods be developed to uncover novel catalysts with diverse activities? Most important, can novel esterolytic catalysts be developed based on currently available catalytic antibody technology to efficiently hydrolyze and detoxicate cocaine?

The answer is unquestionably yes. In fact, cocaine is quite immunogenic; a catalytic antibody was obtained that catalyzed the hydrolysis of cocaine, but the rate enhancement was considerably less than desirable (Landry et al. 1993). The ratio of the catalytic rate constant (k_c) to the spontaneous or water-catalyzed rate constant (k_o)

was 540 and 440 for two catalytic antibodies, respectively, which is considerably less than the rate of hydrolysis of other esters by catalytic antibodies reported in the literature. Thus, immunization of mice with a bovine serum albumin conjugate linked to a phosphonate analog of cocaine (figure 4) afforded antisera that provided monoclonal antibodies after using standard purification procedures. The primary screening for anti-cocaine antibodies was done with an enzyme-linked immunosorbent assay (ELISA) against a phospho-nate analog of cocaine (figure 4). Hybridoma cells immunopositive for anti-phosphonate analog antibodies were next screened for cocaine benzoyl ester hydrolytic activity. The selectivity of the most active anti-cocaine catalytic antibody was shown by the inhibition of the hydrolysis reaction in the presence of the phosphonate transition state analog (figure 4). The K_m values for the two most active anti-cocaine catalytic antibodies were 490 and 1020 M, with k_{cat} values of 0.11 and 0.07 min^{-1}, respectively. These values were significantly less impressive than the values obtained for butyryl cholinesterase, the principal cocaine esterase in human serum (Gatley 1991; Stewart et al. 1978), that provided K_m and k_{cat} values of 38 M and 1.2 min^{-1}, respectively. As stated by Landry and colleagues, a catalytic antibody against cocaine should ideally have a turnover number of at least 2 sec^{-1} and a K_m of approximately 30 M to deactivate cocaine before the molecule partitions into the CNS (Landry et al. 1993). Clearly, room for significant improvement in the catalytic and binding properties of antibodies against cocaine is required before such biocatalysts become part of a human therapeutic regime. In addition, advances in humanizing catalytic antibodies are also needed before artificial esterases will find clinical usefulness in decreasing the serum levels of cocaine during overdose or intoxication in humans.

Structure and Modification of Antibodies

The attachment of drugs and enzymes to antibodies is a common practice in commercial diagnostics, experimental immunoassays, and drug-delivery therapeutic approaches (Chaudhary et al. 1989; Pinchera et al. 1985). Antibody-enzyme conjugates are now being used for targeting cytotoxic pro-drugs to cancerous tissue (Chaudhary et al. 1989). In principle, antibody-enzyme conjugates could offer another possible way

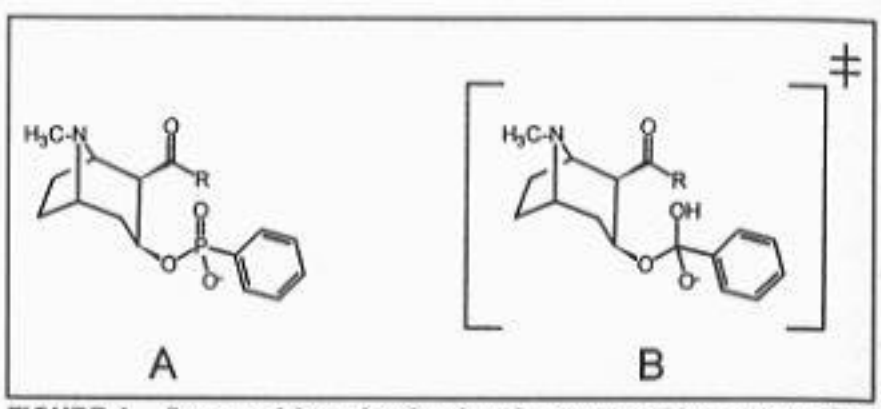

FIGURE 4. *Structural formula of a phosphonate transition state analog of cocaine benzoyl ester hydrolysis (compound A, R = OCH_3) and structural formula of the phosphonate transition state analog of cocaine used in the ELISA assay (Compound A, R = ester tether). Compound B is the structural formula of the putative transition state for benzoyl ester hydrolysis.*

to pharmacologically interdict cocaine or other drugs of abuse. For example, a biocatalyst with cocaine hydrolytic activity could be coupled to an antibody to produce a conjugate with enhanced catalytic and pharmacokinetic properties.

Coupling methods between antibodies and ligands generally rely on chemical modification to selectively introduce the enzyme onto the antibody. Antibodies offer a range of sites for chemical modification. As shown in figure 5, the combining site where antibody-hapten recognition occurs is contained in the Fab fragment. The Fab fragment can be produced by selective proteolytic digestion of the antibody. Thus, a minimal high-affinity binding protein can be obtained by papain digestion of an antibody prepared against the hapten. The variable domains of the light chains (V_L) and heavy chains (V_H) have been frequently documented as having the same antigen-binding activities as the whole antibody (Weir 1986). V_L and V_H fragments can be engineered by molecular biological means to have reduced background (i.e., nonspecific) binding sometimes associated with intact antibodies. In addition, use of the light and heavy chain fragments may be clinically advantageous in that it is possible that they are less immunogenic than the intact antibody but still possess high affinity (Condra et al. 1990). In principle, V_L and V_H fragments are also easier to construct by recombinant methods than intact antibodies because they possess fewer disulfide bonds (Better et al. 1989). However, the stoichiometric expression of V_L and V_H fragments must be linked (Pluckthun 1991). The Fab fragments are

coupled through cysteine residues at the C-terminal end of the heavy chains. Additional cysteines, which are normally involved in interchain disulfide linkages in the hinge region, are retained in a pepsin-generated $F(ab')_2$ fragment (figure 5). Selective coupling of small or large biomacromolecules to $F(ab')_2$ fragments is simple and straightforward. The potential for antibody-enzyme or antibody fragment-enzyme conjugates for use in detoxication of drugs of abuse is essentially unexplored. It is possible that combining the favorable properties of antibody-mediated localization with enzymes or other biocatalysts could provide a new class of detoxication agents.

Anti-Idiotype Antibodies

The immune system discriminates between self and nonself (i.e., foreign) antigen. The foreign antigen can stimulate an immune response, and the immune system sets in motion a steady-state memory lymphocyte which, upon reinfection with the same or closely related antigen, is able to respond with a vigorous immune response. If the foreign antigen is a complementary structure, then one might influence the immune system to recognize the mirror image of the complementary structure. Thus, immunization of an animal with a monoclonal antibody that recognizes the transition state for detoxication of a drug of abuse should elicit an immune response and produce antibodies that resemble the structure of the drug of abuse. The second antibody, the anti-idiotype antibody, thus mimics the transition state for detoxication. Injection of this second antibody into an animal could create a relatively long-term memory effect to give protection against the drug of abuse; the third antibody produced could act as a catalyst to detoxicate the drug (Landry et al. 1993). For example, passive immunization with an anti-cocaine catalytic antibody "could provide a window for appropriate psychosocial and relapse-prevention interventions" (Landry et al. 1993). Of course, researchers are a long way away from passively immunizing humans with catalytic antibodies. However, future use of antibodies in a therapeutic paradigm may provide novel approaches to promoting cessation of use of drugs of abuse as well as in the maintenance of abstinence (Rocio et al. 1995).

Catalytic Antibodies and Catalytic RNA

As discussed above, in vitro selection techniques have been used to isolate biocatalysts useful in the development of catalytic antibodies (Tawfik et al. 1990). While the cocaine catalytic antibodies reported thus

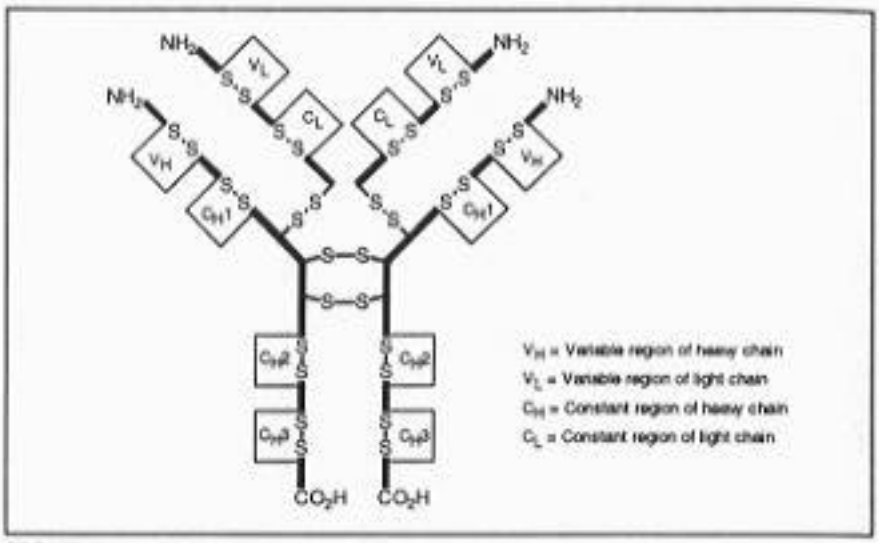

FIGURE 5. *Two-dimensional depiction of an antibody.*

SOURCE: Harlow and Lane 1988.

far do not possess impressive hydrolytic catalytic rate accelerations, the selection technology has nevertheless allowed the discovery of some fundamentals of catalysts that might later support rational design of detoxication catalysts and answer fundamental questions in catalysis (Benner 1993). Catalytic antibody technology relies on the selection of biocatalysts using the enormous diversity of the immune system. Procurement of catalytic species depends on the ability of substrates to chemically bind or transform a target molecule. The key feature of the catalytic antibody is the specificity of the reaction catalyzed. It is interesting to note that the relatively recent advances in catalytic RNA technology have revealed considerable parallels between the concepts of catalytic antibodies and catalytic RNA.

Catalytic RNA

Several structurally and mechanistically distinct classes of catalytic RNAs have been discovered (Altman 1990; Cech 1990; Pace and Smith 1990). Generally, for catalytic RNA reactions reported thus far, RNA and deoxyribonucleic acid (DNA) are substrates and transesterification or hydrolysis of phosphate esters are the reactions catalyzed (Piccirilli et al. 1992). However, binding of amino acids, organic dyes, and other small molecules to RNA suggest that catalytic RNAs may catalyze other reactions (Prudent et al. 1994). Some catalytic RNA molecules are metalloenzymes and require magnesium for their three-dimensional structure and catalytic activity. Like

catalytic antibodies, catalytic RNAs (i.e., ribozymes) catalyze very specific reactions. For example, DNA-cleaving ribozymes with relatively high catalytic efficiency that undergo relatively rapid turnover and operate in a highly specific manner have been discovered (Tsang and Joyce 1994). Future studies may show that catalytic RNA has utility as sequence-specific DNA endonucleases. The specificity stems from the first step in the ribozyme reaction which is due to specific Watson-Crick base-pairing interactions (Mueller et al. 1993). In every ribozyme thus far examined, a template region (named the internal guide sequence) (Seiwert and Stuart 1994) has been observed near the 5'-end of the molecule that forms Watson-Crick base pairs with the target RNA substrate. The 3'-hydroxy of a base at the other end of the ribozyme attacks the phosphodiester bond within the ribozyme-bound substrate complex. A transesterication reaction occurs that results in cleavage of the substrate and ligation of the 3'-portion of the substrate (Tsang and Joyce 1994). Just like in the case of enzyme- or catalytic antibody-catalyzed reactions, the rate depends upon substrate binding affinity and the intrinsic catalytic rate parameters. For example, in ester hydrolysis there is a hyperbolic dependence on the concentration of the ribozyme: at low concentration of catalyst the rate of hydrolysis is first order, while at high concentration of catalyst the reaction rate is indepen-dent of ribozyme concentration (Piccirilli et al. 1992). This type of saturation or Michaelis-Menten kinetic behavior is typical of ribozymes and is completely analogous to the enzyme-substrate complex observed for enzymes and catalytic antibodies. Like other biocatalysts, RNA-cleaving ribozymes are highly specific for RNA and catalyze hydrolysis with high catalytic efficiency, undergo rapid turnover, and operate in a highly selective manner. In addition, inhibition of ribozyme catalysis has been observed for substrate analogs; like enzymes and other biomacro-molecules, changes in ribozyme solvation and conformation have been observed when the inhibitor binds (Piccirilli et al. 1992).

Ribozymes may be developed in the future to act as aminoacyl tRNA synthetases. Ribozymes might be developed to selectively break acyl bonds to oligonucleotides or might also be engineered to selectively charge oligonucleotides with amino acids. Again, due to the specificity of Watson-Crick base-pair interactions, oligonucleotide catalysts may find use in the therapeutics of human disease. Highly selective ribozyme endonucleases may be created to perform sequence-specific cleavages of potential therapeutic value. The ratio of a toxic dose to a therapeutic dose (i.e., [toxic dose]/[therapeutic dose]) presumably will be very high for an effective ribozyme

primarily due to the high specificity of oligonucleo-tides (i.e., containing 15 to 17 nucleotides) that should have a unique sequence relative to the entire human genome (Stein and Cheng 1993). In principle, suitable oligonucleotides should be able to interfere in a sequence-specific manner with processes such as translation of messenger RNA (mRNA) into a specific receptor or other protein. If the synthesis of the target protein encoded by the mRNA is required for susceptibility to drug addiction, it may be possible to develop new therapeutic strategies based on elaboration of ribozymes or oligonucleotides targeted to inhibit or inactivate key biological processes.

SUMMARY

Currently there is a significant amount of information about the way biocatalysts from animals detoxicate and bioactivate drugs of abuse. In some cases, biotransformation data concerning drugs of abuse obtained from animal systems are analogous to the human situation, but in many cases the data are not. Clearly, significant work needs to be done with human biocatalysts to define a role in the biotransformation of drugs of abuse and to relate the work that has already been done in animals. New metabolic pathways will likely be discovered that may link drug metabolism to addiction liability or drug susceptibility in humans.

New design and selection technologies are providing the basis to allow the discovery of new biocatalysts that may be useful in the detoxication of drugs of abuse in humans. Fundamentally new approaches using biocatalysts including rationally engineered enzymes, catalytic antibodies, catalytic antibody fragments, ribozymes, oligonucleotides, and other biomacromolecules may provide basic information that may later support the rational design of biocatalysts, which may in turn provide the basis for designing detoxication catalysts for drugs of abuse.

NOTES

1. In this chapter, nicotine or nicotine metabolites shall all be assumed to have the (*S*) stereochemistry at the benzylic center.

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