# Human Liver Cocaine Carboxylesterases

William F. Bosron, Robert A. Dean, Monica R. Brzezinski, and Evgenia V. Pindel

The stimulant effects of cocaine are relatively short lived, as evidenced by the increase in heart rate that peaks at about 60 minutes after cocaine administration and declines thereafter (Farre et al. 1993). This duration of effect is largely due to the rapid hydrolysis of cocaine to two major deesterified metabolites that appear in serum and urine, benzoylecgonine and ecgonine methyl ester (figure 1). Neither of these deesterified metabolites is active as a stimulant when administered peripherally, even at high doses (Spealman et al. 1989; Misra et al. 1975). The rapid distribution half-life of cocaine from an intravenous (IV) dose is about 10 minutes and the elimination half-life of cocaine is 50 to 80 minutes (Jeffcoat et al. 1989). Hence, the duration of the stimulant action of cocaine is limited by its rate of hydrolysis to inactive metabolites.

The time course for toxic effects of cocaine also appears to be dependent on drug metabolism. For example, almost one-third of the deaths related to cocaine overdose occur about 2 to 5 hours after drug administration (Wang and Carpentier 1994), a time period equal to about two half-lives of cocaine in serum. Sudden cardiac death is one of the acute toxic effects of cocaine overdose, but the mechanism is not fully understood. Direct effects of cocaine on the electrophysiological properties of isolated heart muscle preparations have been shown in vitro (Wang and Carpentier 1994). Cocaine and some of its metabolites have also been shown to be potent vasoconstrictors. Hence, decreased blood flow and hypoxia of cerebral arterioles may be important contributors to cerebral infarction, hemorrhage, and developmental abnormalities, especially in newborn infants exposed to cocaine in utero (Kutrh et al. 1993; Covert et al. 1994). Cocaine-induced hepatotoxicity has been reported in both humans and rodents and the extent of hepatic necrosis appears to be dependent, in part, on the content of microsomal cocaine-metabolizing enzymes (Roth et al. 1992). Hence, the duration and magnitude of the desired stimulant effects, as well as the undesired toxic effects of cocaine use, appear to depend on the content and activity of enzymes responsible for cocaine metabolism.

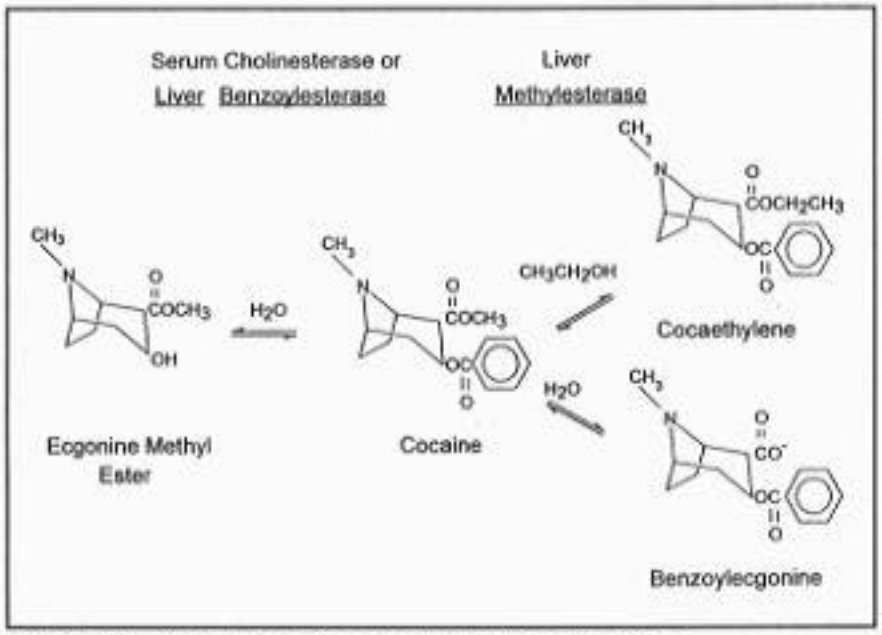


**FIGURE 1.** *Structures of cocaine metabolites formed by carboxylesterases.*

The hydrolysis of the benzoyl ester group of cocaine to ecgonine methyl ester and benzoate (figure 1) is catalyzed by serum cholinesterase (Stewart et al. 1977), also called pseudocholinesterase or butyrylcholines-terase, and a human liver carboxylesterase (Dean et al. 1991). The hydro-lysis of the methyl ester group of cocaine to benzoylecgonine and methanol (figure 1) occurs spontaneously in aqueous solutions at neutral to alkaline pH. However, the half-life of cocaine at pH 7.2 and 40% is about 5 hours (Garrett and Seyda 1983), which is substantially greater than the half-life of cocaine in vivo, about 1 hour (Jeffcoat et al. 1989). A carboxylesterase was recently identified in human liver that catalyzes the hydrolysis of this methyl ester of cocaine to form benzoylecgonine and methanol (Dean et al. 1991). In the presence of ethanol, the enzyme also catalyzes the ethyl transesterification of cocaine to form cocaethyl-ene plus methanol (Dean et al. 1991).

## CHARACTERIZATION OF A HUMAN LIVER COCAINE BENZOYL ESTER HYDROLASE

An enzyme that catalyzes the hydrolysis of cocaine to ecgonine methyl ester and benzoate was purified from human autopsy liver by ion-exchange and affinity chromatography and gel filtration. The enzyme was purified approximately 8,700-fold and about 150 micrograms (g) of enzyme were obtained from 70 grams (g) of human liver. The enzyme catalyzed the hydrolysis of cocaine to ecgonine methyl ester and benzoate and the hydrolysis of methylumbelliferylacetate to methylumbelliferone. The Michaelis constant ($K_M$) for cocaine was 0.7 millimolars (mM) and the turnover number ($k_{cat}$) was 7.6 $min^{-1}$. The enzyme was inhibited by phenylmethylsulfonyl fluoride, diazinon, and eserine. The enzyme is a monomer of approximately 60 kilodalton (kDa) subunit mass. It has an isoelectric point (pI) of approximately 4.9 and is a glycoprotein.

The physiological levels of cocaine can reach a maximum of about 0.3 micromolar (M) in serum immediately after cocaine administration (Jeffcoat et al. 1989). These levels are much less than the $K_M$ of the liver benzoyl esterase (0.7 mM). Hence, the enzyme will obey first-order kinetics, where activity equals $k_{cat}/K_M$, called the catalytic efficiency, times the cocaine concentration. The catalytic efficiency of the liver benzoyl esterase, 11 $min^{-1}$ $mM^{-1}$, is similar to human serum cholinesterase, 7 $min^{-1}$ $mM^{-1}$, another enzyme that catalyzes the hydrolysis of the benzoyl group of cocaine. The content and kinetic properties of serum cholinesterase and liver benzoyl esterase need to be evaluated to determine which enzyme has the greater capacity for hydrolysis of cocaine in human.

The catalytic efficiency of the liver cocaine benzoyl esterase and serum cholinesterase is about 50 times that of a catalytic antibody prepared to an analog of the putative cocaine benzoyl esterase transition-state structure, 0.22 $min^{-1}$ $mM^{-1}$ (Landry et al. 1993). The objective for preparing the catalytic antibody was to provide a reagent that could bind and enhance the clearance of cocaine (Morell 1993). It was proposed that such a reagent could be used to blunt the desire to use cocaine. However, it is not clear whether a cocaine benzoyl esterase that is more efficient than the native serum or liver enzymes can be created by catalytic antibody technology. Perhaps the clearance of cocaine might be increased by inducing the native microsomal benzoyl esterases present in liver.

## CHARACTERIZATION OF A HUMAN LIVER COCAINE METHYL ESTER HYDROLASE

An enzyme that catalyzes the hydrolysis of the methyl ester of cocaine (figure 1) was purified to homogeneity by ionexchange and hydrophobic interaction chromatography and gel filtration (Brzezinski et al. 1994). Approximately 3 milligrams (mg) of enzyme were purified from 70 g of human liver obtained at autopsy. The purified carboxylesterase catalyzes the hydrolysis of cocaine to benzoylecgonine and methanol and the hydrolysis of methylumbelliferyl acetate to methylumbelliferone and acetate. The enzyme is a trimer of approximately 59 kDa subunits. It has a pI value of 5.8 and it is a glycoprotein. Fourteen different tryptic and *S. aureus* V8 peptides were purified by high performance liquid chromatography (HPLC) and their amino acid sequences were determined. After searching the amino acid and translated DNA sequences deposited in GenBank, two identical matches were found that correspond to nonspecific carboxylesterase cDNAs from human liver and lung (Long et al. 1991; Riddles et al. 1991; Munger et al. 1991).

In addition to the hydrolysis of cocaine, the purified human liver cocaine methyl ester hydrolase also catalyzed the ethyl transesterification of cocaine with ethanol to form cocaethylene and methanol as shown in figure 1 (Dean et al. 1991; Brzezinski et al. 1994). Both the hydrolytic and the ethyl transesterification reactions increased as the two activities were analyzed in protein fractions obtained during the enzyme purifi-cation by column chromatography. This suggests that the separate activities are catalyzed by the same enzyme. The $K_M$ values for cocaine and ethanol of the purified enzyme at pH 7.3 were 116 M and 43 mM, respectively. The carboxylesterase also catalyzes the formation of ethyloleate from oleic acid and ethanol (Tsujita and Okuda 1992; Brzezinski et al. 1994). Other hydrolases or ester transferases have been reported to catalyze similar substrate "ethylation" reactions. For example, an isozyme of glutathione-S-transferase will also catalyze the fatty acid ethyl-ester synthase reaction, leading to the formation of ethyloleate from oleic acid and ethanol (Bora et al. 1989). Also, phospholipase D catalyzes the transphosphatidylation of phosphatidylcholine with ethanol to form phosphatidylethanol (Kobayashi and Kanfer 1987). The active site requirements and kinetics of the hydrolases or transferases that catalyze these ethylation reactions are not well understood. The elucidation of mechanisms and active site structures for enzyme-catalyzed

ethylation reactions should contribute to the basic understanding of alcohol-drug interactions.

The coabuse of cocaine and alcohol is all too common (Grant and Harford 1990), and is thought to result from the enhanced euphoria and sense of well-being experienced when cocaine is taken with alcohol (Farre et al. 1993). Unfortunately, coabuse of alcohol and cocaine results in an increased health risk (Farre et al. 1993). The formation of cocaethylene under defined experimental conditions in rats after administration of alcohol and cocaine and the cellular and organ toxicity of cocaethylene are discussed by Dean and colleagues elsewhere in this volume.

## STRUCTURE-REACTIVITY RELATIONSHIPS FOR COCAINE CARBOXYLESTERASES

The human liver cocaine methyl and benzoyl carboxylesterases catalyze the hydrolysis of the acetyl group of 4-methylumbelliferyl acetate with specific activities of 6.8 and 140 mol/(min ß mg protein), respectively. With cocaine as substrate, however, the cocaine methyl and benzoyl estereases exhibit lower specific activities, but show absolute specificity for the methyl ester group and the benzoyl ester group, respectively. A high specificity for binding of cocaine derivatives to proteins or catalytic specificity for metabolic reactions is frequently observed. For example, Gatley reported that the benzoyl group of benzoylecgonine was not hydrolyzed by serum cholinesterase (Gatley 1991). Additionally, Gatley observed that serum cholinesterase exhibited about a 2,300-fold higher relative activity with the unnatural (+) isomer of cocaine than the natural (-) isomer (Gatley 1991).

The determination of structure-reactivity relationships of cocaine-metabolizing enzymes and binding proteins represents an important direction for research in cocaine metabolism. Binding site structures could be determined directly by protein X-ray crystallography and computerized modeling of cocaine binding. Such structure-reactivity studies will provide valuable information regarding potential drug or metabolite interactions with these important cocaine metabolizing enzymes and binding proteins.

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## ACKNOWLEDGMENTS

This chapter was prepared with support from the National Institute on Drug Abuse grant DA-06836.

AUTHORS

William F. Bosron, Ph.D.
Professor
Department of Biochemistry and Molecular Biology
Department of Medicine

Robert A. Dean, Ph.D., M.D.
Associate Professor
Department of Pathology and Laboratory Medicine
Department of Biochemistry and Molecular Biology

Monica R. Brzezinski, B.S.
Graduate Student
Department of Biochemistry and Molecular Biology

Evgenia V. Pindel, Ph.D.
Postdoctoral Fellow in Biochemistry and Molecular Biology

Indiana University School of Medicine
Medical Sciences Building
635 Barnhill Drive
Indianapolis, IN 46202-5122

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