The endogenous activity of butyrylcholinesterase (BChE; E.C.3.1.1.8) in plasma substantially influences the rate at which cocaine is metabolized (Carmona et al., 1996). It has been demonstrated that inhibition of endogenous BChE activity by tetraisopropyl pyrophosphoramide (Iso-OMPA, a selective plasma cholinesterase inhibitor), followed by a single bolus cocaine challenge, significantly increases cocaine lethality in mice and rats (Hoffman et al., 1992b). Limited circumstantial evidence suggests that endogenous BChE activity is inversely correlated with the severity of acute cocaine toxicity in humans. Individuals experiencing more severe medical problems after cocaine use tend to have lower plasma BChE activity than those experiencing less severe problems (Devenyi, 1989; Hoffman et al., 1992a; Om et al., 1993). These results together suggest that alterations in cocaine metabolism were reversed by a specific BChE inhibitor. In the second, in vivo, experiment, exogenously administered BChE reduced peak cocaine concentrations when given to anesthetized squirrel monkeys. Finally, incubation of cocaine with added BChE in human plasma in vitro resulted in a decrease in cocaine half-life similar to that observed with squirrel monkey plasma. The magnitude of the decrease in cocaine half-life was proportional to the amount of added BChE. Together, these results indicate that exogenously administered BChE can accelerate cocaine metabolism in such a way as to potentially lessen the behavioral and toxic effects of cocaine. Therefore, BChE may be useful as a treatment for cocaine addiction and toxicity.

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Abbreviations used are: BChE, butyrylcholinesterase; BE, benzoylecgonine; EME, ecgonine methyl ester; Iso-OMPA, tetraisopropyl pyrophosphoramide; GC-MS, gas chromatography-mass spectrometry.

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presence of additional metabolic pathways in the intact animal may alter the overall metabolic effect of added BChE. This study also provides a direct comparison of the in vitro/in vivo action of BChE on cocaine metabolism. Finally, we evaluated the effects of BChE addition on the in vitro metabolism of cocaine in human plasma. For this experiment, the amount of BChE was varied to look for a possible concentration-dependent metabolite profile.

Materials and Methods

Squirrel Monkey (In Vitro). The plasma from six male squirrel monkeys with no recent drug exposure was pooled and used for the in vitro assay. The following conditions were investigated: 1) saline alone (1.65 ml); 2) monkey plasma alone (1.6 ml); 3) monkey plasma (1.6 ml) + BChE (100.0 μl) + BChE inhibitor (50.0 μl). The added BChE inhibitor consisted of 27 mM phosphostigmine plus a saturated solution of Iso-OMPA. Each sample received 100.0 μl of cocaine solution (for a final calculated concentration of 1297 ng/ml), and the final volume was adjusted to 1.85 ml with the addition of appropriate amounts of phosphate buffer and saline. BChE solution was prepared in phosphate buffer (0.1 M, pH 7.4) to yield a final concentration of approximately 1000 U/ml (100 U/100 μl). Thus, the final BChE concentration per sample was 54 I.U./ml. The pH of plasma-containing samples was measured to be 7.81; nonplasma 7.40. Each sample was placed in a 37°C water bath. A zero-timepoint aliquot (100 μl) was removed from each sample just before the addition of BChE. Aliquots (100 μl) for cocaine analysis were then removed from each sample at 5, 15, 30, 60, 90, 120, and 240 min. Aliquots were collected into tubes containing a saturated solution of sodium fluoride in 10% acetic acid. Aliquots were immediately placed on dry ice followed by storage at −30°C until analysis by solid phase extraction followed by gas chromatography-mass spectrometry (GC-MS).

Squirrel Monkey (In Vivo). Male monkeys (n = 4) were housed individually in light-, temperature-, and humidity-controlled rooms. Fresh drinking water was available ad libitum. Daily food intake was controlled to maintain a steady body weight (750 –1250 g). All monkeys had been in previous drug control conditions throughout the entire 240-min sampling period (Fig. 1c). In plasma alone, there were gradual decreases in BE, with a cocaine half-life of 43 min. The addition of BChE dramatically accelerated cocaine metabolism, with the cocaine half-life decreasing to 5.4 min. The addition of the BChE inhibitor completely blocked the cocaine concentration remaining above 1000 ng/ml under saline conditions. BChE has a disappearance half-life of approximately 72 h in rhesus monkeys (Broomfield et al., 1998), should yield an approximate final BChE activity of 140 mU/ml of plasma (based on squirrel monkey blood volume of approximately 70 ml/kg, and plasma volume about half blood volume). BChE was administered as a 30-min pretreatment before cocaine (3.0 mg/kg). This dose, chosen to be comparable with the dose used in our prior studies (Carmona et al., 1998), should yield an approximate final BChE activity of 140 U/ml of plasma (based on squirrel monkey blood volume of approximately 70 ml/kg, and plasma volume about half blood volume). BChE has a disappearance half-life of approximately 72 h in rhesus monkeys (Broomfield et al., 1991), suggesting that BChE activity would have remained stable over the 2-h sample collection period. Three animals were tested under both conditions, whereas the fourth was tested only with BChE due to catheter difficulties. Both enzyme and cocaine were administered as single bolus i.v. injections over 5 s. Equal volumes of saline were immediately injected after the administration of BChE or cocaine to flush residuals from the catheter. Blood samples were drawn (from the right catheter) 5 min before and 1, 2.5, 5, 10, 15, 30, 60, and 120 min after cocaine administration for assessment of plasma cocaine and metabolite concentrations. Catheters were kept patent after each sampling with infusions of heparin (0.3 μl). Samples were transferred into heparinized collection tubes containing a saturated sodium fluoride solution (in 10% acetic acid). The plasma was separated by centrifugation at 3000 RPM for 10 min, and then stored at −90°C until GC-MS analysis.

Human (In Vitro). Pooled human plasma (obtained from drug-naive volunteers) was used for the assay. The following conditions were investigated: 1) saline (1.0 ml); 2) plasma (1.0 ml); 3) plasma (1.0 ml) + BChE (25 μl); 4) plasma (1.0 ml) + BChE (50 μl); and 5) plasma (1.0 ml) + BChE (100 μl). Cocaine solution (100.0 μl) was added to each sample and the final volume was brought to 1.85 ml with equivalent amounts of phosphate buffer and saline to maintain a consistent pH. The concentration of the added BChE solution was 1000 U/ml, yielding added doses of 13.5, 27, and 54 U/ml of BChE. The final concentration of cocaine in the sample was 1143 ng/ml. Each sample was placed in a 37°C water bath. A zero-timepoint aliquot (100 μl) was removed from each sample just before BChE addition. Similar aliquots (100 μl) were then removed from each sample at 5, 15, 30, 60, and 90 min. Aliquots were immediately placed on dry ice followed by storage at −30°C until GC-MS analysis. A saturated sodium fluoride (in 10% acetic acid) solution was added to each tube as a preservative before collection.

Chemicals and Materials. Drug standards and materials for use in the analytical assay were obtained from the following sources: cocaine hydrochloride (National Institute on Drug Abuse Intramural Research Program, Baltimore, MD; Mallinckrodt, St. Louis, MO); BE tetrahydrate (Research Biochemicals International, Natick, MA); horse serum-derived BChE, EME hydrochloride, [2 H 3 ]cocaine HCl, [2 H 3 ]BChE tetrahydrate, [2 H 3 ]EME hydrochloride and Iso-OMPA (Sigma Chemical Company, St. Louis, MO); N-O-bis (trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical Co, Rockford, IL), Clean Screen solid phase extraction columns (ZSDUA020; United Chemical Technologies, Bristol, PA). Methanol, methylene chloride, 2-propanol, and acetonitrile were HPLC grade and all other chemicals were reagent grade. Horse serum-derived BChE was provided as 1000 U/mg protein, and may have contained an unknown amount of protein without BChE activity (personal communication, Sigma Chemical Co.).

Analytical Procedure for Cocaine and Metabolites. In vitro mixtures and plasma specimens were analyzed for cocaine, BE, and EME by a modified procedure (Cone et al., 1994). Briefly, plasma specimens were mixed with deuterated internal standard solution and acidified with sodium acetate buffer (2 M; pH 4.0) followed by centrifugation (3000 rpm for 10 min) and solid phase extraction. Cocaine analyses were eluted with freshly prepared elution solvent (methylene chloride/2-propanol/ammonium hydroxide; 80:20:2, v/v/v) and the eluent was evaporated under nitrogen in a 40°C water bath and reconstituted in 20 μl of acetonitrile. The samples were transferred to autosampler vials and combined with 20 μl of derivatizing reagent (N,O-bis (trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane). The vials were sealed and incubated at 80°C for 30 min. Duplicate matrix-matched calibration curves across the concentration range of 3.1 to 1000 ng/ml for cocaine, BE, and EME were included in each batch of specimens. The limit of detection of the assay was approximately 1 ng/ml for all analytes. Control samples containing all analytes at concentrations of 100 and 500 ng/ml were processed in duplicate with each run. Accuracy of control measurements was within 20% for all analytes. Gas chromatography-mass selective detection was performed with a Hewlett-Packard (Wilmington, DE) 5971 mass selective detector interfaced to a Hewlett-Packard 5890A gas chromatograph with an autosampler (HP7673A). A 1-μl aliquot of the derivatized sample was injected in the splitless mode onto an HP-1 fused silica capillary column (12 m × 0.2 mm i.d., 0.33 μm film thickness). The MS was operated in the selected ion monitoring mode.

Data Analysis. Plasma elimination half-lives for cocaine were determined from log cocaine concentration versus time plots. The data were fitted by linear regression. The half-lives were then determined by the following relationship: $k_{el} = \frac{-\log_{2}}{t_{1/2}}$, where $k_{el}$ is the cocaine elimination rate constant and $t_{1/2}$ is the cocaine elimination half-life.

Results

Squirrel Monkey In Vitro. Minimal spontaneous hydrolysis of cocaine to BE was observed for the saline control conditions. The cocaine concentration remained above 1000 ng/ml under saline control conditions throughout the entire 240-min sampling period (Fig. 1a). Cocaine metabolism was observed in the plasma-alone condition, with a cocaine half-life of 43 min. The addition of BChE dramatically accelerated cocaine metabolism, with the cocaine half-life decreasing to 5.4 min. The addition of the BChE inhibitor completely blocked the effect of the added and endogenous BChE. Cocaine concentrations in the plasma sample with added inhibitor approximated those of the saline samples.

For the saline control condition, EME was not detected at any time point (Fig. 1b); however, there was a gradual increase in BE, with a peak concentration of approximately 150 ng/ml observed during the final sampling period (Fig. 1c). In plasma alone, there were gradual
increases in both EME and BE. The addition of BChE reduced the peak BE concentration by nearly 3-fold, and nearly doubled EME concentrations (Fig. 1b). The addition of the BChE inhibitor reduced the formation of EME, as EME was not detected throughout the sampling period (assay limit of detection = 1 ng/ml). BE concentrations for the inhibitor condition approximated those of the saline condition.

**Squirrel Monkey In Vivo.** Pretreatment with BChE (30 min before cocaine administration) produced an immediate and sustained reduction in plasma cocaine concentration (when compared with controls). Peak cocaine concentrations occurred at 1 min and were reduced nearly 3-fold in the four monkeys receiving BChE pretreatment compared with controls (Fig. 2a). The reduction in plasma cocaine concentration was paralleled by an increased formation of EME, with higher peak concentrations observed in monkeys pretreated with BChE (146.2 ± 32.8 ng/ml; mean ± S.E.) compared with controls (66.3 ± 6.7 ng/ml; mean ± S.E.; Fig. 2b). Concentrations of BE were not significantly different for the two conditions (Fig. 2c). Monkeys receiving BChE did not show changes in either physiological (i.e., eating, water consumption, gastrointestinal disturbances) or behavioral functioning (lowered behavioral activity or hyperactivity) after recovery from anesthesia.

**Human In Vitro.** Cocaine concentration remained fairly stable in the saline control condition, as only small amounts of BE were detected. Cocaine metabolism was observed in the plasma-alone condition, with concentrations decreasing at a half-life of 100.3 min. The addition of 25, 50, and 100 U of BChE accelerated cocaine metabolism in an activity-related manner, and dramatically reduced the half-life of cocaine to 37.6 min (63% reduction), 21.5 min (79% reduction), and 13.1 min (87% reduction), respectively. The elimination rate constant for the 0, 25, 50, and 100 U BChE conditions were 0.41, 1.11, 1.93, and 3.18 h\(^{-1}\), respectively.

The addition of BChE also changed the concentrations of cocaine metabolites in an activity-related manner. In the saline sample, no EME was detected at any time point, but there was a progressive increase in BE concentrations, with the mean peak concentration (83.4 ng/ml) observed at the final sampling time point. In plasma alone, EME and BE concentrations increased over time. Peak EME concentrations (485.5 ng/ml) and peak BE concentrations (80.9 ng/ml) were observed at the end of the 90-min sampling period. The addition of 25, 50, and 100 U of BChE dose dependently lowered peak BE concentrations (from 80.9 to 48.7, 28.6 ng/ml, and to levels less than the limit of quantitation), and increased EME concentrations (from 485.5 to 744, 945.9, and 921.4 ng/ml), respectively.

**Discussion**

Each of the in vitro experiments demonstrates that the addition of BChE to plasma enhances cocaine metabolism. Humans and monkeys tend to have differing baseline BChE activity, with a range of 1.27 to 3.66 U/liter in squirrel monkeys and 2.11 to 7.13 U/liter in humans (Carmona et al., 1996; Washington et al., 1996). Despite these differences in endogenous activity, relatively comparable decreases in cocaine half-life were observed for each species in vitro. The addition of 100 U of BChE to each plasma type led to an 86.9% reduction in the human and an 86.5% reduction in the monkey half-life of cocaine. Additionally, the effect of added BChE was dose-related in human plasma, with the greatest decrease in cocaine half-life occurring at the highest dose. This degree of consistency and dose dependence suggests that the added BChE is solely responsible for accelerating the metabolism of cocaine in plasma in vitro. Any reduction in the plasma concentration of cocaine would be expected to decrease the amount of cocaine in systemic circulation and thus, available to enter the brain, thereby potentially reducing the behavioral and toxic effects of cocaine.
The in vitro addition of the BChE inhibitor (Iso-Ompa) completely reversed the effect of added BChE on cocaine metabolism and EME production in squirrel monkey plasma. In fact, with the addition of the BChE inhibitor, cocaine metabolism was more similar to the saline condition than to plasma. This suggests that the inhibitor blocked the effect of not only the added BChE, but also the endogenous BChE. These findings provide support for a role of endogenous plasma BChE in cocaine metabolism in both nonhuman primates and humans.

BChE also altered the metabolic profile of cocaine. Although BE and EME were both observed in plasma alone, with the addition of BChE, EME concentrations were increased whereas BE concentrations decreased. This effect was also dose-dependent. From a physiological standpoint, decreased concentrations of BE have some important implications. First, previous studies have shown that BE can mediate potent vasoconstrictive effects (Madden and Powers, 1990; Erzouki et al., 1993), and lower the cocaine-induced seizure threshold (Konkol et al., 1976). In contrast, it has been suggested that EME has vasodilative properties that might oppose cocaine-induced vasoconstriction (Madden and Powers, 1990). Therefore, increasing EME production may be beneficial in reducing the overall toxic effects of cocaine.

Although the effects of BChE added to plasma in vitro were remarkably consistent, effects were somewhat different with the in vivo experiments. For example, although the addition of BChE dramatically reduced the concentration of BE in vitro, BE concentrations in vivo were not changed. The concentration of EME was increased slightly in vivo, although probably not to the degree that it was in vitro. This observed variance between in vivo and in vitro studies is probably due in part to other mechanisms that can aid in the elimination of cocaine from the body in vivo. For example, hepatic mechanisms of metabolism not present in vitro, including liver carboxyl esterases (Dean et al., 1991; Brzezinski et al., 1994; Pindel et al., 1997), would contribute to the different pattern of results observed with the in vivo study compared with the in vitro study. Nevertheless, the reduced concentrations of cocaine observed in the in vivo study after BChE administration also support the potential utility of BChE as a treatment for cocaine toxicity and addiction (Gorelick, 1997). The reduction in the peak concentration of cocaine in vivo also indicates that the effect of added BChE may be rapid enough to reduce even the initial behavioral effects of cocaine. Additional enhancement of BChE activity may be achieved by using mutant forms of BChE (e.g., Xie et al., 1999), which are more capable of hydrolyzing cocaine.

The findings of this study have important implications for future work in the treatment of cocaine toxicity and addiction. First, the clear comparability of the effects of added BChE between monkey and human plasma suggests that work in this area can be productively pursued in animal species, and still be relevant for humans. In fact, our previous in vitro work in rat plasma demonstrated that adding BChE to rat plasma in doses comparable with those used in the present study had similar effects (Carmona et al., 1998). Furthermore, the in vivo work supported the in vitro studies, indicating that BChE can alter the metabolism of cocaine even in the intact animal. In fact, the rapid reduction in the peak cocaine concentration suggested that this effect may be rapid enough to alter even the immediate behavioral effects of cocaine. This work also suggests that the effects observed in previous studies, reduction in the behavioral (Carmona et al., 1998) and toxic (Mattes et al., 1997) effects of cocaine after in vivo administration of BChE, probably do result from enhanced metabolism of cocaine. Overall, these studies provide additional support for the hypothesis that exogenous BChE can be used as a treatment for both cocaine toxicity and addiction.

**References**


![Monkey In Vivo Results](image-url)
Protection by butyrylcholinesterase against organophosphorus poisoning in nonhuman pri-
Brezinski MR, Abraham TL, Stone CL, Dean RA and Boston WF (1994) Purification and
characterization of a human liver cocaine carboxylesterase that catalyzes the production
of benzoylecgonine and the formation of cocaethylene from alcohol and cocaine. Biochem
Carmona GN, Baum I, Schindler CW, Juffer R, Cone EJ, Belendik GW and
Gorelick DA (1996) Plasma butyrylcholinesterase activity differs significantly in rhesus and
Carmona GN, Schindler CW, Shoaib M, Juffer R, Cone EJ, Goldberg SR, Greig NH, Yu QS and
Gorelick DA (1998) Attenuation of cocaine-induced locomotor activity by butyrylcholinest-
Cone EF, Hillsgrove M and Darwin WD (1994) Simultaneous measurement of cocaine, coca-
ethylene, their metabolites, and “crack” pyrolysis products by gas chromatography-mass
Dean RA, Christian CD, Sample RHB and Boston WF (1991) Human liver cocaine esterases:
168.
Erozouki HK, Baum I, Goldberg SR and Schindler CW (1993) Comparison of the effects of
Gorelick DA (1997) Enhancing cocaine metabolism with butyrylcholinesterase as a treatment
Hoffman RS, Henry GC, Wax PM, Weisman RS, Howland MA and Goldfrank LR (1992b)
Decreased plasma cholinesterase activity enhances cocaine toxicity in mice. J Pharmacol Exp
Ther 263:698–702.
cholinesterase protects against cocaine toxicity in mice. J Toxicol Clin Toxicol 34:259–266.
Konkol RJ, Erickson BA, Doer JK, Hoffman RG and Madden JA (1976) Seizures induced by
Madden JA and Powers RH (1990) Effect of cocaine and cocaine metabolites on cerebral arteries
Therapeutic use of butyrylcholinesterase for cocaine intoxication. Toxicol Appl Pharmacol
Medical complications of cocaine: Possible relationship to low plasma cholinesterase enzyme.
Am Heart J 125:1114–1117.
Pindel EV, Kedilvitiy NY, Abraham TL, Brezinski MR, Zhang J, Dean RA and Boston WF
(1997) Purification and cloning of a broad substrate specificity human liver carboxylesterase
255.
improved cocaine hydrolase: The A328Y mutant of human butyrylcholinesterase is 4-fold