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Intravenous butyrylcholinesterase administration and plasma and brain levels of cocaine and metabolites in rats

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Abstract

Butyrylcholinesterase is a major cocaine-metabolizing enzyme in humans and other primates, catalyzing hydrolysis to ecgonine methylester. Increasing butyrylcholinesterase activity may be a treatment for cocaine addiction. We evaluated the effect of 30-min pretreatment with horse-derived butyrylcholinesterase (5–15,000 U i.v.) or with the selective butyrylcholinesterase inhibitor cymserine (10 mg/kg i.v.) on the metabolism of cocaine (17 mg/kg i.p.) in anesthetized rats. Venous blood samples were collected for two hours after cocaine administration and later assayed for cocaine and metabolites by gas chromatography/mass spectroscopy. Whole brains were collected after the last blood sample and similarly assayed. Butyrylcholinesterase significantly increased plasma and brain ecgonine methylester levels and decreased cocaine plasma half-life from 26.2 min (saline) to 16.4 min (15,000 U). Butyrylcholinesterase had no significant effect on plasma or brain cocaine or benzoylecgonine levels. Cymserine had no effect on any variable. These findings suggest that butyrylcholinesterase treatment may have benefits in enhancing cocaine metabolism and in increasing levels of ecgonine methylester, which may have a protective action against cocaine.

Keywords: Cocaine; Butyrylcholinesterase; Ecgonine methylester; Cymserine; Benzoylecgonine; (Rat)

1. Introduction

Butyrylcholinesterase (EC 3.1.1.8) is a major cocaine-metabolizing enzyme in primates, including humans (Carmona et al., 1996; Inaba, 1989), catalyzing the hydrolysis of cocaine to ecgonine methylester (Matsubara et al., 1984). Enhancement of butyrylcholinesterase activity has been proposed as a treatment approach for cocaine addiction (Gorelick, 1997). The hypothesis is that enhanced enzyme activity would degrade enough cocaine to significantly lower drug concentration at its sites of action, thereby reducing cocaine's effects.

Treatment with exogenous butyrylcholinesterase enhances cocaine metabolism in vitro in rodent, monkey, and human plasma (Browne et al., 1998; Carmona et al., 1996, 2000) and in vivo in rodents, cats, and monkeys (Mattes et al., 1997; Carmona et al., 2000), resulting in increased concentrations of ecgonine methylester.

As might be expected, pretreatment with natural or enhanced variants of butyrylcholinesterase reduces the acute behavioral effects of cocaine in rodents (Carmona et al., 1998; Koetzner and Woods, 2002; Lynch et al., 1997; Mattes et al., 1997; Sun et al., 2002). Conversely, inhibition of butyrylcholinesterase activity reduces cocaine metabolism and decreases ecgonine methylester concentrations (Carmona et al., 2000; Hoffman et al., 1992;

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Kambam et al., 1992, 1993). However, butyrylcholinesterase inhibition has been variously reported as enhancing (Hoffman et al., 1992), reducing (Gao and Brimijoin, 2004; Hoffman et al., 1996; Kambam et al., 1992), or having no effect on (Kambam et al., 1993; Knuepfer and Gan, 1999) the acute cardiovascular effects of cocaine (perhaps depending on the dose and rate of cocaine administration).

We are aware of only two published studies evaluating the effects of butyrylcholinesterase treatment on brain concentrations of cocaine. Mattes et al. (1997) found that i.v. administration of human butyrylcholinesterase (7.8 mg/kg) to anesthetized rats 1 min after 175 mg/kg i.p. or 2 min after 30 mg/kg i.p. of cocaine reduced brain cocaine concentrations by 80% 4 min later and by 30% 45 min later, respectively. Koetzner and Woods (2002) found that i.v. administration of horse butyrylcholinesterase (14,100 or 25,000 U/kg) to anesthetized mice 52 min before 30 mg/kg i.p. of cocaine reduced brain cocaine concentrations by 25% 8 min later. Neither study measured concentrations of cocaine metabolites.

We report here the effect of enhancement (with exogenous butyrylcholinesterase) or inhibition (with cymserine) of butyrylcholinesterase activity on plasma and brain cocaine and metabolite levels in anesthetized rats.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats weighing approximately 275 g were housed individually with fresh drinking water and food available ad libitum. They were maintained on a 12:12 h light–dark cycle, with lights on at 7:00 a.m. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Aging Intramural Research Program and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Procedures

On the day of the experiment, rats were anesthetized with ketamine (50–100 mg/kg). Catheters were then inserted into the jugular and saphenous veins. A baseline sample of blood was taken from the saphenous catheter. Rats were divided into 7 groups and injected intravenously (i.v.) through the jugular catheter with saline, cymserine (10.0 mg/kg), or 5, 50, 500, 5000 or 15000 IU horse-serum-derived butyrylcholinesterase as a bolus over approximately 5 s. Cymserine is a specific inhibitor of butyrylcholinesterase (Yu et al., 1999). The dose used was expected to inhibit about two-thirds of plasma butyrylcholinesterase activity. An equal volume of saline was injected immediately after the administration of butyrylcholinesterase or cymserine to flush residuals from the catheter. Thirty minutes later, an intraperitoneal (i.p.) injection of cocaine (17.0 mg/kg) was administered. The cocaine dose was chosen on the basis of previous work in our laboratory as producing a reliable, but non-maximal, increase in behavioral activity. All drugs were dissolved in saline and given in a volume of 1.0 ml/kg.

Blood samples (200–400 μl) were drawn via the indwelling saphenous catheter at 2, 5, 10, 30, 60, 90 and 120 min after cocaine administration for assessment of plasma cocaine and metabolite concentrations. Catheters were kept patent after each sampling with an infusion of heparin (0.05 ml). Samples were transferred into heparinized collection tubes containing a saturated sodium fluoride solution (in 10% acetic acid) to inhibit cocaine metabolism. The plasma was separated by centrifugation at 15,000 g for 10 min, and then stored at −90 °C for subsequent gas chromatography/mass spectroscopy analysis. Following the last blood sample, some rats were decapitated under anesthesia and whole brains removed for analysis of cocaine and metabolites. Brains were stored at −80 °C until analysis.

2.3. 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); horse serum-derived butyrylcholinesterase (Sigma Chemical Co, St. Louis, MO); cymserine hydrochloride (Dr. Nigel H. Greig, National Institute on Aging, Baltimore, MD); Clean Screen solid phase extraction columns (ZSDAU020; United Chemical Technologies, Bristol, PA). Methanol, methylene chloride, 2-propanol, and acetonitrile were HPL grade and all other chemicals were reagent grade. Horse serum-derived butyrylcholinesterase was provided as 1000 U/mg protein, and may have contained an unknown amount of protein without butyrylcholinesterase activity (personal communication, Sigma Chemical Co.).

2.4. Analytical procedure for cocaine and metabolites

In vitro mixtures and plasma specimens were analyzed for cocaine, benzoylecgonine, and ecgonine methylester by modification of a published 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 (15,000 g for 10 min) and solid phase extraction. Cocaine analytes 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 then 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, benzoylecgonine, and ecgonine methylester 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 duplicates 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 5980A 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 mass spectrometer was operated in the selected ion-monitoring mode.

2.5. Data analysis

Only those animals with data from the last blood collection time point were included in the analysis. Area-Under-the-Curve (AUC) was calculated for plasma concentrations of cocaine, benzoylecgonine and ecgonine methylester. Peak levels and time to peak were determined by observation of the data. Two-way analysis-of-variance (ANOVA) was performed for plasma levels with time as a within-subjects factor and treatment condition as a between-subjects factor. One-way ANOVAs with a single between-subjects treatment factor were performed with AUC, peak, time to peak, and cocaine half-life values. Paired comparisons between the saline group and another group were done as post hoc tests within the ANOVA. Plasma elimination half-lives for cocaine were determined for each subject from the log-cocaine concentration versus time plots. The peak of the cocaine blood level curve was assumed to be time zero; the data were fit by linear regression. The half-lives were then determined by the following relationship: $k_{el} = 2.203 \times \text{slope}$; $t_{1/2} = 0.693/k_{el}$, where k_{el} is the cocaine elimination rate constant and $t_{1/2}$ is the cocaine elimination half-life. Average half-life was calculated from these values for each group.

3. Results

3.1. Plasma levels of cocaine and metabolites

Neither cocaine nor its metabolites were detected in any of the baseline plasma samples. Cocaine levels rose rapidly after injection ($n = 4–7$/group, $F_{5,155} = 31.3$, $P < 0.001$ for time factor), with the peak typically occurring 5–10 min following the injection (Fig. 1A). Butyrylcholinesterase treatment had no significant overall effect on cocaine plasma levels ($F_{6,31} = 1.6$, $P = 0.19$ for treatment factor; $F_{30,155} = 1.0$, $P = 0.46$ for time × treatment interaction), peak cocaine plasma level ($F_{6,31} = 1.7$, $P = 0.15$), time to peak level ($F_{6,31} = 0.9$, $P = 0.48$), AUC ($F_{6,31} = 1.5$, $P = 0.20$), or cocaine half-life (Fig. 2) ($F_{6,30} = 1.7$, $P = 0.16$). Post hoc tests showed that both the 15,000 U ($n = 6$, 16.4 ± 2.3 min) and 5000 U ($n = 6$, 16.8 ± 2.0 min) butyrylcholinesterase treatment groups had significantly shorter cocaine half-lives than the saline group ($n = 7$, 26.2 ± 2.2 min) (Fig. 2) ($F_{1,30} = 5.2$, $P = 0.03$, and $F_{1,30} = 5.3$, $P = 0.03$, respectively).

In contrast to plasma cocaine levels, there was a statistically significant, dose-dependent butyrylcholinesterase effect on plasma ecgonine methylester levels (Fig. 1B) and AUC (data not shown), with higher butyrylcholinesterase doses generating higher ecgonine methylester levels ($F_{6,29} = 10.3$, $P < 0.001$ for treatment effect; $F_{30,145} = 2.3$, $P < 0.01$ for treatment × time interaction) and larger AUCs ($F_{6,29} = 11.1$, $P < 0.001$). Likewise, the peak ecgonine methylester level was significantly ($F_{6,29} = 9.3$, $P < 0.001$) higher for both the 15,000 and 5000 butyrylcholinesterase treatment groups. The time to peak ecgonine methylester level averaged around 30–60 min for all treatment groups (Fig. 1B) and was not significantly altered by butyrylcholinesterase treatment ($F_{6,29} = 2.1$, $P = 0.08$). Increased ecgonine methylester levels were still present 2 h after cocaine administration (Fig. 1B), a time when cocaine levels themselves were close to zero (Fig. 1A).

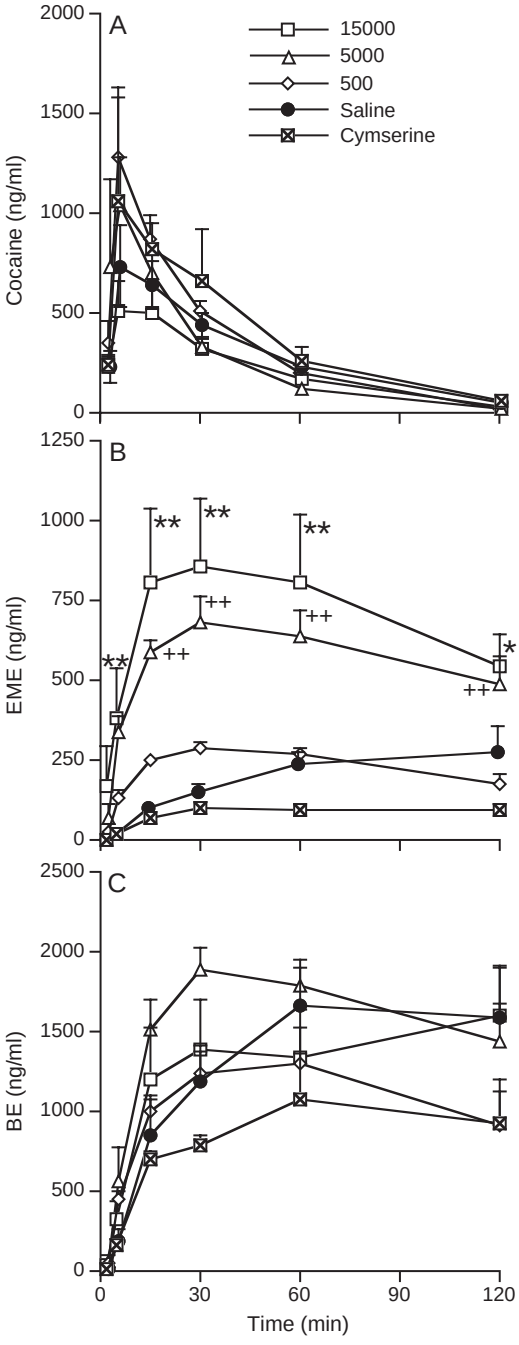

Fig. 1. Time course for plasma levels of cocaine (A), ecgonine methyl ester (B), and benzoylecgonine (C). All animals were given an i.p. injection of 17 mg/kg cocaine at time zero. Thirty minuntes prior to the cocaine injection, separate groups (4–7 animals per group) of rats were given i.v. injections of butyrylcholinesterase (500, 5000 or 15 000 U), saline, or 10 mg/kg cymserine. Error bars are +S.E.M. Data for the two lowest butyrylcholinesterase doses (5, 50 U) are omitted for the sake of clarity (the two curves were similar to the curve for the saline group). $^{**}P < 0.01$ and $^{*}P < 0.05$ for saline vs. BChE 15,000 U $^{++}p < 0.01$ for saline vs. BChE 5000 U.

Plasma benzoylecgonine levels were not significantly affected by butyrylcholinesterase treatment ($F_{30,145} = 1.2$, $P = 0.2$ for time × treatment interaction) (Fig. 1C).

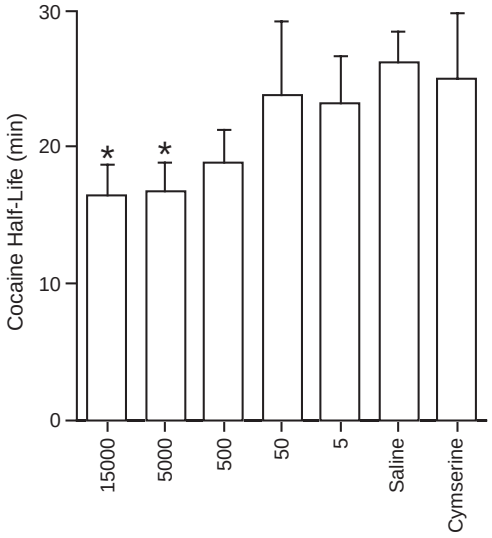

Fig. 2. Cocaine plasma half-life for groups (4–7 animals per group) of rats treated with i.v. injections of butyrylcholinesterase (5, 50, 500, 5000 or 15,000 U), saline, or 10 mg/kg cymserine 30 min prior to an i.p. injection of 17 mg/kg cocaine. Half-life was calculated for each individual rat and then a mean half-life was determined. Error bars are +S.E.M. *$p<0.05$ vs. saline.

Post hoc analyses showed that treatment with the butyrylcholinesterase inhibitor cymserine ($n=4$) did not significantly alter peak plasma levels, AUC (data not shown), or time to peak levels for either cocaine (Fig. 1A), ecgonine methylester (Fig. 1B), or benzoylecgonine (Fig. 1C), nor did it alter cocaine half-life (Fig. 2).

3.2. Whole brain levels of cocaine and metabolites

Whole brain levels of cocaine ($n=3–5$/group, $F_{5,21}=1.2$, $P=0.35$) and benzoylecgonine ($F_{5,21}=1.1$, $P=0.39$) were not significantly affected by either butyrylcholinesterase or cymserine treatment (Fig. 3A, C). Ecgonine methylester brain levels were significantly ($F_{5,21}=14.0$, $P<0.001$) increased by the highest butyrylcholinesterase dose (Fig. 3B).

4. Discussion

In this study, exogenous butyrylcholinesterase in vivo produced a dose-dependent increase in rat plasma and brain ecgonine methylester concentrations (Figs 1B, 3B), with the two highest doses, 15,000 U and 5000 U, also shortening cocaine half-life (Fig. 2). This extends to the brain the previously reported ecgonine methylester increase in plasma (Carmona et al., 2000; Mattes et al., 1997) and suggests that peripherally administered butyrylcholinesterase can influence brain cocaine metabolism. Both peripherally and intracranially administered ecgonine methylester have been reported to protect against toxic and behavioral effects of cocaine in rodents (Hoffman et al., 2004; Schuelke et al., 1996). The present findings suggest that butyrylcholinesterase might have a beneficial effect not only by shortening cocaine half-life or reducing cocaine levels, but also by increasing levels of a metabolite, ecgonine methylester, that may reduce toxic and behavioral effects of cocaine.

The two highest butyrylcholinesterase doses, 15,000 U and 5000 U, significantly shortened cocaine half-life,

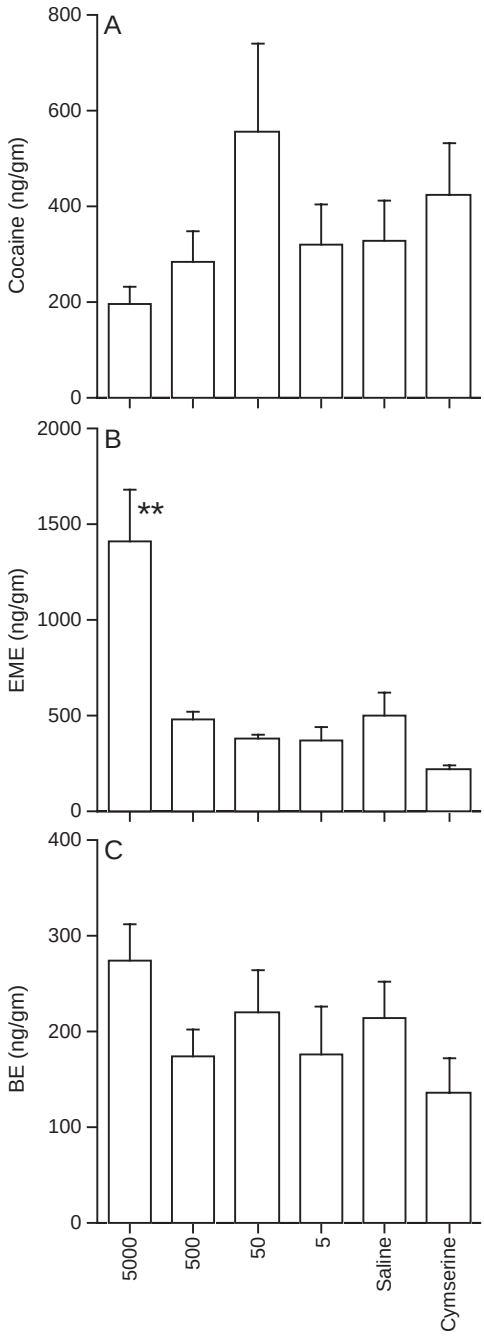

Fig. 3. Brain concentrations of cocaine (A), ecgonine methyl ester (B), and benzoylecgonine (C) for rats treated with i.v. injections of butyrylcholinesterase (5, 50, 500, or 5000 U), saline, or 10 mg/kg cymserine 30 min prior to an i.p. injection of 17 mg/kg cocaine. Rats were sacrificed and brain removed immediately following the last blood sample collection, which was 120 min after the cocaine injection. Error bars are +S.E.M. There were 3–5 rats per group. **$p<0.01$ vs. saline.

compared with saline, but did not significantly alter cocaine plasma levels. This finding suggests that clinical use of butyrylcholinesterase may need higher doses to reduce peak cocaine plasma levels than to shorten cocaine half-life. However, other studies in rodents and cats have reported dose differences in the influence of butyrylcholinesterase on cocaine's pharmacodynamic effects vs. on pharmacokinetics, i.e., doses that significantly reduced cocaine's physiological or behavioral effects produced no change in cocaine half-life and only modest, non-dose-dependent decreases in cocaine concentrations (Koetzner and Woods, 2002; Mattes et al., 1997; Sun et al., 2002). These differences may be related, in part, to the fact that the primary endogenous enzymes for cocaine metabolism in non-primates are not butyrylcholinesterase, but carboxylesterases, which catalyze the hydrolysis of cocaine to benzoylecgonine (Estevez et al., 1977; Warner and Norman, 2000). The activity of these endogenous carboxylesterases may have outweighed the effect of any exogenous butyrylcholinesterase. Human studies of the cocaine–butyrylcholinesterase interaction, using human enzyme, are needed to determine the dose of enzyme that will significantly reduce cocaine's effects.

The dose of cymserine used should have inhibited about two-thirds of plasma butyrylcholinesterase activity. The lack of effect of cymserine may have been due to the very minor role that butyrylcholinesterase plays in cocaine metabolism in non-primates. Cymserine has no inhibitory effect on carboxylesterases (Yu et al., 1999).

As expected, butyrylcholinesterase had no significant effect on benzoylecgonine levels (Fig. 1c), because benzoylecgonine is not a product of butyrylcholinesterase-catalyzed cocaine hydrolysis.

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