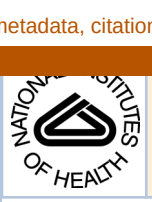

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Modification of pharmacokinetic and abuse-related effects of cocaine by human-derived cocaine hydrolase in monkeys

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Abstract

Although substantial research effort has focused on developing pharmacological treatments for cocaine abuse, no effective medications have been developed. Recent studies show that enzymes that metabolize cocaine in the periphery, forestalling its entry into the brain, can prevent cocaine toxicity and its behavioral effects in rodents. Here we report on effects of one such enzyme (Albu-CocH) on the pharmacokinetic and behavioral effects of cocaine in squirrel monkeys. Albu-CocH was developed from successive mutations of human butyrylcholinesterase (BChE) and has 1000-fold greater catalytic activity against cocaine than naturally occurring BChE. Pharmacokinetic studies showed that Albu-CocH (5 mg/kg) had a half-life of 56.6 hours in squirrel monkeys. In these studies, plasma levels of cocaine following i.v. 1 mg/kg cocaine were reduced two hours after administration of Albu-CocH, whereas plasma levels of the cocaine metabolite ecgonine methyl ester were increased. These effects were still evident 72 hrs following Albu-CocH administration. In behavioral experiments in monkeys, pretreatment with 5 mg/kg Albu-CocH dramatically decreased self-administration of a reinforcing dose of i.v. cocaine (30 μg/kg/injection) for over 24 hours. Pretreatment with 5 mg/kg Albu-CocH also attenuated the reinstatement of extinguished cocaine self-administration by an i.v. priming injection of cocaine (0.1 or 0.3 mg/kg) and, in separate studies, attenuated the discriminative stimulus effects of cocaine. The ability of Albu-CocH to attenuate the abuse-related effects of cocaine in squirrel

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Authors Contribution

CWS, ZJ, LS-T, SY, JB and SRG were involved with the design of the research. CWS collected blood for the pharmacokinetic study, ZJ and GHR collected data for the self-administration studies and JB collected data for the drug discrimination study. HH analyzed the pharmacokinetic data. DL, DW and VR developed the assays and provided the Albu-CocH blood levels and Albu-CocH antibody analysis. CWS wrote the original manuscript draft and all authors critically reviewed content and approved the final version for publication.

monkeys indicates that further investigation of BChE mutants as potential treatment for cocaine abuse and toxicity is warranted.

Keywords

cocaine; hydrolase; self-administration; reinstatement; discrimination; squirrel monkeys

Introduction

Despite the fact that substantial research effort has focused on developing a pharmacological treatment for cocaine abuse, no clearly effective medications have been developed (Vocci and Elkashef, 2005). Since actions within the central monaminergic receptor system are thought to mediate the abuse of cocaine, much of the research in this area has focused on the use of direct and indirect dopamine receptor agonists and antagonists as treatments (Karila et al., 2008). A different pharmacological approach might be to restrict cocaine's access into the brain, perhaps by sufficiently degrading the cocaine molecule in the periphery before it can enter the central nervous system. Along these lines, passive or active immunization to cocaine could be used to sequester cocaine in the periphery. This approach involves treating individuals with a cocaine-like molecule that has been linked to a larger protein that will evoke an immunological response. For example, Fox et al. (1996) developed a cocaine vaccine that was synthesized by conjugating norcocaine to the immunogenic carrier protein bovine serum albumin. Treatment with this compound produced an immunologic response that was sufficient to antagonize the self-administration of cocaine in rats. A similar approach has also been successful in reducing the subjective effects of cocaine in humans (Haney et al., 2010).

A related approach is based on the observation that naturally occurring plasma butyrylcholinesterase (BChE) metabolizes cocaine primarily to ecgonine methyl ester (EME), which has minimal behavioral and physiological effects (Gorelick, 1997). Presumably, such a compound might be capable of metabolizing cocaine in the blood before it even has a chance to enter the central nervous system. In this regard, Carmona et al. (2000, 2005) showed that horse-serum derived BChE can enhance the metabolism of cocaine in vivo in rats and primates, and in human plasma. Horse-serum derived BChE was also capable of antagonizing the locomotor activating effects of cocaine in rats (Carmona et al., 1998).

Although such earlier studies provided proof of concept, the relatively low activity makes it unlikely that BChE itself could be used successfully in the treatment of either cocaine abuse or toxicity. Therefore, a number of investigators have focused on developing compounds capable of metabolizing cocaine more efficiently than native BChE. For example, Cooper et al. (2006) reported on a cocaine esterase (CocE) found in the soil around the coca plant. This esterase, which is more efficient than BChE at metabolizing cocaine, can both protect against cocaine-induced lethality in rats and antagonize ongoing cocaine self-administration in rats (Cooper et al., 2006, Brim et al., 2010, Collins et al., 2009). Other investigators have focused on developing mutants of native BChE that have greater activity against cocaine and a longer duration of action. Pan et al. (2005) developed a quadruple mutant from human BChE (A199S/S287G/A328W/Y332G, CocH) that has 1000-fold greater catalytic activity against cocaine than the naturally occurring BChE (Gao et al., 2008). To obtain a form of this mutated BChE that had favorable pharmacokinetics, the mutant BChE was fused at its C terminus with human serum albumin. This type of procedure has been used to extend the half-life of other biologicals (Duttaroy et al., 2005) and the albumin fused quadruple mutant (Albu-CocH) retained high catalytic efficiency and had a half-life of over 8 h in rats

(Brimijoin et al., 2008). Like the previously-studied esterases, the Albu-CocH compound was capable of antagonizing both cocaine toxicity and the reinstatement of cocaine self-administration by a priming injection of cocaine in rats (Brimijoin et al., 2008, Carroll et al., 2011). The present studies were conducted to further evaluate this compound in several abuse-related procedures (cocaine self-administration, cocaine primed reinstatement, psychomotor stimulant drug discrimination) in nonhuman primates. Pharmacokinetic analysis was also carried to out to establish the duration of Albu-CocH in the blood and Albu-CocoH's effects on the plasma levels of cocaine and its metabolites. Albu-CocH decreased the reinforcing, reinstatement-related, and discriminative-stimulus effects of cocaine, findings that may be readily translatable into the human population.

Materials and Methods

Subjects

Twelve adult male squirrel monkeys (*Saimiri sciureus)* weighing 0.8 to 1.2 kg were used as subjects. Three monkeys for cocaine self-administration had permanently indwelling venous catheters that were protected by a nylon vest. Details of catheter implantation are given in Justinova et al. (2003). Subjects had unrestricted access to water and were fed a daily amount to maintain stable free-feeding body weight (Lab Diet 5045, PMI Nutrition International, Richmond, IN supplemented with fresh fruits, vegetables, and Banana Softies, Bio-Serv, Frenchtown, NJ). All monkeys were individually housed in a humidity- and temperature-controlled room and were provided with environmental enrichment daily. The animal care facilities were fully accredited by AAALAC and all experiments were approved by the NIDA Intramural Research Program Animal Care and Use Committee.

Pharmacokinetic Studies

Five squirrel monkeys were used to determine the duration of Albu-CocH in the blood and to determine the effect of Albu-CocH on the plasma levels of cocaine and its metabolites. No gross behavioral effects were ever observed in any monkey for this or any of the studies described below following the administration of 5 mg/kg Albu-CocH. Three monkeys (#547, #3434 and #53B) were given Albu-CocH (5 mg/kg, i.m.) and, two hours later, received an i.v. injection of cocaine (1 mg/kg). Cocaine was also administered 72 and 96 hours after Albu-CocH administration. Blood was also collected for Albu-CocH determination before its administration and 24, 72, 96 and 336 hrs after injection. The 336 hr sample was also used to determine Albu-CocH immunogenicity. Briefly, samples (approximately 0.4 ml) were collected from a femoral vein and placed in serum separation tubes that were maintained at room temperature for at least 1 hour before centrifugation. Centrifugation yielded at least 0.2 ml of serum that was frozen at approximately -70° C for later analysis. Also, femoral blood samples (0.4 ml) also were collected 5 and 30 min following each cocaine injection for determination of levels of cocaine and metabolites. Samples were placed in EDTA tubes spiked with an esterase inhibitor (diisopropylfluorophosphate, DFP) that were maintained on wet ice until they were centrifuged at 2-8° C within 45 min of collection. The resulting plasma was placed in polypropylene tubes and stored at -70° C for later analysis. Two monkeys (#548, #27B) served for control purposes and were injected with Albu-CocH vehicle and, 2 hours later, 1 mg/kg cocaine (i.v.). Blood was collected 5 and 30 min after the cocaine injection for cocaine analysis as described above. Monkeys #547, #548, #3434 and #538 had a history of nicotine self-administration followed by cocaine self-administration. All had been drug free for over one year prior to the start of the current study.

Cocaine Self-Administration

Three squirrel monkeys (#5045, #39B and #70F7) were trained to self-administer i.v. cocaine (30 μg/kg/injection) in daily 60-min sessions. These same 3 monkeys were used for reinstatement testing as detailed below. Details of the self-administration training procedure can be found elsewhere (Justinova et al., 2003). All the monkeys had extensive experience with cocaine self-administration. The monkeys were trained to respond under a fixed-ratio 10 (FR10) schedule of cocaine injections, i.e., the completion of 10 lever-press responses in the presence of a green stimulus light initiated a 200 msec i.v. injection of cocaine into the indwelling venous catheter. The beginning of the daily session was signaled by the illumination of the green stimulus light. The injection of cocaine was accompanied by a 2-sec flash of a yellow stimulus light and the offset of the green stimulus light for a 60-sec timeout during which responding had no programmed consequences (TO 60-sec). At the end of the timeout, the green light was re-illuminated and the FR10 schedule was again in effect.

Following the establishment of stable cocaine self-administration and reliable extinction of responding following saline substitution, Albu-CocH (5 mg/kg, i.m.) or its vehicle (i.m.) were given 2 hours prior to a Monday self-administration session. During drug testing, i.v. cocaine self-administration or saline extinction were studied in blocks of 5 consecutive daily sessions. Following each drug test or saline substitution, responding for 30 μg/kg cocaine was reestablished for at least 5 days. Following reinstatement testing (see below), the dose of cocaine available for self-administration was lowered to 10 μg/kg/injection and the effect of Albu-CocH (5 mg/kg, i.m.) was again determined.

Reinstatement of Cocaine Self-Administration

Saline was substituted for cocaine in monkeys that reliably self-administered 30 μg/kg cocaine. Responding rapidly decreased following saline substitution. Reinstatement tests were conducted by administering cocaine (0.3 mg/kg, i.v.), 5 min prior to the session to measure cocaine-reinstated self-administration responding. Initially, the vehicle of Albu-CocH was given 2 hrs before a test session. The priming dose of cocaine was also given prior to sessions of saline availability 48 and 96 hours later, but without any further injections of the Albu-CocH vehicle. In subsequent test sessions, Albu-CocH (5 mg/kg, i.m.) was given 2 hours prior to a second sequence of 3 reinstatement tests with cocaine. Following these tests, the monkeys were returned to baseline conditions under which 30 μg/kg/injection cocaine was available for self-administration. Subsequently, saline was again substituted for cocaine and a second series of reinstatement tests were conducted in a similar manner except that 0.1 mg/kg i.v. cocaine was given prior to reinstatement sessions.

Drug Discrimination

Four monkeys (#12-98, #20-99, #21-99, #85-04) were trained to discriminate i.m. injections of methamphetamine (0.056 mg/kg) from vehicle under an FR 10 schedule of stimulus termination. All of the monkeys had extensive experience with the methamphetamine drug discrimination procedure prior to the Albu-CocH testing. Cocaine fully substitutes for methamphetamine and pretreatment with Albu-CocH prior to cocaine testing was used to determine the ability of Albu-CocH to antagonize the discriminative stimulus effects of cocaine. Albu-CocH was given prior to methamphetamine testing to determine the specificity of that effect. Monkeys sat in chairs prepared with two response levers and visual stimulus lights as described above; additionally, the distal segment of the subject's tail was secured in a stock and lay under two electrodes. Daily sessions began with the illumination of green stimulus lights. During their illumination, delivery of a mild current (200 msec; <3mA) across the electrodes was programmed to occur every 10 sec. Completion of 10 lever-press responses turned off the stimulus lights and initiated a 50 sec timeout. If the response requirement was not met within 30-sec, the stimulus lights were automatically

terminated and the 50-sec timeout was initiated. Discrimination training began when responding was reliably maintained by the termination of the visual stimulus. During training, sessions began 5 min after i.m. pretreatment and comprised 10 presentations of the FR10;TO 50 sec stimulus-termination schedule. Completion of the FR10 on one of the two levers after an i.m. injection of saline terminated the visual stimulus. Completion of the FR10 schedule on the other lever terminated the visual stimulus after i.m. injection of 0.056 mg/kg methamphetamine. Once monkeys reliably discriminated methamphetamine from vehicle (>90% correct responses for 5 consecutive sessions), test sessions were conducted where responding on either lever was correct (i.e., completion of the FR10 on either lever turned off the visual stimulus). Subsequently, the terminal procedure was introduced, and daily sessions consisted of 1-4 components of 10 trials, with each component separated by 10 min. Under this procedure, subjects could be injected 5-min prior to each component for cumulative dose-effect determinations during test sessions. The effects of cumulative injections of i.m. methamphetamine and cocaine were established in all subjects prior to testing with Albu-CocH or its vehicle. Cumulative doses of cocaine were then tested 2, 24 and 48 hours following a single injection of 5 mg/kg Albu-CocH. The dose-effect function for methamphetamine was then tested 2 hours following 5 mg/kg Albu-CocH.

Drugs

Albu-CocH was supplied by TEVA Pharmaceutical (Netanya, Israel) in a frozen solution at a concentration of 30 mg/ml. Details for the preparation of the protein can be found in Gao et al. (2008). Briefly, Albu-CocH is a C-terminally truncated (E1-V529) and mutant (A199S, S287G, A328W,Y332G) form of BChE (accession number gi:116353), fused to the N-terminus of human serum albumin (gi:28592). This monomeric protein was expressed in Chinese hamster ovary cellsstably transfected with the gene for Albu-CocH. The clonal cell line was adapted for suspension and serum-free growth in a bioreactor and was grown for 10 days prior to harvest of the conditioned culture media. Protein was initially captured on Blue Sepharose and further purified using DEAE Sepharose followed by Q-HP Sepharose ion exchange chromatography. The albumin–cocaine hydrolase fusion (Albu-CocH) retains a catalytic efficiency with cocaine (k_{cat} = 2,700 min^{-1}, K_m = 2 μM) that is 1,000-fold higher than natural human BChE, and it exhibits a half-life of 8 h after i.v. injection in rats (Gao et al. 2008). The supplied solution was diluted to 15 mg/ml with vehicle (10 mM sodium phosphate, 200 mM mannitol, 60 mM trehalose dihydrate, 0.01% polysorbate 80) and then given to the monkeys i.m. in a volume of 0.33 ml/kg. Cocaine (NIDA, Baltimore, MD) was dissolved in saline. In self-administration, cocaine was given in volumes of 0.2 ml/injection. In reinstatement, cocaine was injected i.v. in volume 1 mg/ml. In discrimination, cocaine was injected i.m. (0.3 ml/kg). Methamphetamine was also dissolved in saline and given in a volume of 0.3 ml/kg.

Data Analysis

The blood level data were analyzed separately for each metabolite and time point (5 or 30 min). A one-way analysis-of-variance (ANOVA) was performed with follow-up Dunnett tests using the results of monkeys treated with vehicle as the control. The self-administration data were analyzed separately for response rate or injections following either vehicle treatment or Albu-CocH treatment. Baseline data were included in the analysis by taking the average of the last 3 days for the immediately preceding baseline condition (cocaine self-administration or cocaine self-administration following vehicle pretreatment). A within-subjects ANOVA was then performed with follow-up contrasts (Wilkinson, 1992) comparing the 5 days following vehicle or Albu-CocH with the baseline. For reinstatement a two-way ANOVA was performed for both response rate and injections, with time post pretreatment as the within-subjects factor (2, 48 and 96 hrs) and pretreatment (vehicle or Albu-CocH) as the between subjects factor. Follow-up contrasts compared vehicle and

Albu-CocH at each time point (2, 48 or 96 hrs). For drug discrimination, the percent drug lever responding and response rates in test sessions were compared to control (methamphetamine alone) values in a one-way ANOVA with follow-up Dunnett tests. The pre Albu-CocH results with methamphetamine and cocaine discrimination were used as the control values.

Results

Pharmacokinetic Studies

No Albu-CocH was detected in samples collected prior to Albu-CocH administration. Figure 1 shows serum levels of Albu-CocH for 3 monkeys at 24, 72, 96 and 336 hours following an i.m. injection of 5 mg/kg Albu-CocH. Blood levels were remarkably consistent across animals, with differences in levels only clearly evident after 2 weeks. The average half-life of Albu-CocH was estimated to be 56.6 hours (range 45.5 – 65.5 hours).

Cocaine was administered 2, 72 and 96 hours after administration of a single 5 mg/kg Albu-CocH injection. Figure 2 shows the plasma levels of cocaine (top panel), ecgonine methyl ester (EME, middle panel) and benzoylecgonine (BZ, bottom panel) at 5 and 30 min following each cocaine injection. Two hours following administration of Albu-CocH cocaine plasma levels were significantly reduced at both 5- and 30-min following the cocaine injection when compared to animals who were injected with cocaine following vehicle ($F_{3,7} = 7.34$, $p < 0.05$ and $F_{3,7} = 14.4$, $p < 0.005$ respectively). Seventy-two hours following Albu-CocH administration, cocaine levels were still significantly below control levels 30-min post cocaine. The effects of Albu-CocH were not significant 96 hours following Albu-CocH at either the 5 or 30 min post-cocaine time point. The effects of Albu-CocH on EME levels were similar, but in the opposite direction to those of cocaine. Plasma levels of EME were elevated at both the 5- and 30-min time points post-cocaine administration at 2 hr following Albu-CocH dose ($F_{3,7} = 5.6$, $p < 0.05$ and $F_{3,7} = 33.3$, $p < 0.001$). Plasma levels of EME remained significantly elevated at 72 hours following Albu-CocH for the 30 min post-cocaine time point. While plasma levels of BZ appeared to be slightly reduced 2 hr following Albu-CocH, these effects were not significant.

Cocaine Self-Administration

Albu-CocH reduced self-administration behavior maintained by 30 μg/kg/inj cocaine. The right panels of Figure 3 show response rate (top panel) and injections (bottom panel) over consecutive days. The first five days show baseline responding. Saline was then substituted for cocaine and both rate ($F_{5,10} = 37.0$, $p < 0.001$) and injections ($F_{5,10} = 188.7$, $p < 0.001$) were significantly reduced for the 5 days of substitution when compared to the average of the last 3 days of the immediately preceding baseline (left panels days 3-5) period. Following a 5 day return to baseline, monkeys were treated with vehicle and given 5 days of self-administration training. When compared to the average of the last 3 days of the immediately preceding baseline period (lefts panels days 3-5), vehicle treatment had no effect on injections ($F_{5,10} = 2.6$, $p = 0.10$). An overall effect of days was observed for response rate ($F_{5,10} = 4.6$, $p < 0.05$), however, follow-up contrasts failed to reveal any significant change in responding from baseline on any of the 5 days following vehicle treatment. Following vehicle treatment, monkeys were given a single injection of 5 mg/kg Albu-CocH prior to day 21 and cocaine self-administration was tracked for 5 days. Significant changes from the average of the last 3 days of the previous baseline (left panels days 18-20) were observed for both response rate ($F_{5,10} = 4.6$, $p < 0.05$) and injections ($F_{5,10} = 14.1$, $p < 0.001$). Follow-up contrast revealed that response rate was different from baseline on days 1-4 following treatment and injections were significantly different from baseline on day 1 and 4 following treatment.

Following reinstatement testing (see below), monkeys were returned to self-administration, but at a lower cocaine dose (10 μg/kg/inj). Decreasing the maintenance dose decreased rate of responding and number of injections (Figure 3, right panels). Following baseline stability, monkeys were given a vehicle injection and self-administration continued for 5 days. Neither response rate nor injections significantly changed following vehicle injection compared to the average of the last 3 days on baseline (right panels days 1-3). Albu-CocH (5 mg/kg) was then given and cocaine self-administration continued for an additional 5 days. Both response rate and injections were lower 2 hrs following the Albu-CocH injection when compared to baseline (days 6-8), but these effects failed to reach significance.

Reinstatement of Cocaine Self-Administration

In monkeys that responded for 30 μg/kg/inj cocaine, substituting saline for cocaine led to a rapid decrease in responding. The Albu-CocH vehicle was then given i.m. and 2, 48 and 96 hrs later 0.3 mg/kg cocaine i.v. was given 5 min before a saline substitution session. Priming with cocaine led to a reinstatement of cocaine self-administration responding as shown in the black bars in the left panels of Figure 4. In the presence of 5 mg/kg Albu-CocH given i.m., however, reinstatement of responding by 0.3 mg/kg cocaine i.v. was significantly reduced (compare black and white bars) at the 2 hr time point for both response rate ($F_{1,4}$ = 16.9, $p < 0.05$) and number of injections ($F_{1,4}$ = 34.9, $p < 0.001$). Monkeys were subsequently tested in an identical manner with 0.1 mg/kg cocaine as the reinstatement dose. While reinstatement to the lower cocaine dose was more variable, a similar pattern of results was observed with the effect of 5 mg/kg Albu-CocH again significant at 2 hrs for both responses ($F_{1,4}$ = 15.2, $p < 0.05$) and injections ($F_{1,4}$ = 129.7, $p < 0.001$). Reinstatement at 48 and 96 hrs did not differ significantly in the presence or absence of 5 mg/kg AlbuCocH.

Drug Discrimination

In 4 monkeys that were trained to discriminate 0.056 mg/kg methamphetamine from saline, cocaine fully substituted for methamphetamine at a dose of 0.3 mg/kg (Figure 5, top panel). When 5 mg/kg Albu-CocH was given 2 hrs prior to the determination of the cocaine dose-effect function, 0.3 mg/kg cocaine failed to generalize to the methamphetamine cue ($F_{12,38}$ = 8.8, $p < 0.001$) and a 3-fold higher i.m. dose of cocaine (1.0 mg/kg) was required to produce partial substitution (~70%) for methamphetamine. Twenty-four hrs following Albu-CocH, the 0.3 mg/kg dose of cocaine now partially substituted (~70%) for the methamphetamine cue, and 1.0 mg/kg now fully generalized to methamphetamine. By 48 hrs following Albu-CocH, the 0.3 mg/kg dose again fully generalized to the methamphetamine cue. In contrast to its effects on cocaine, pretreatment with Albu-CocH 2 hrs prior to the determination of the methamphetamine dose-effect function did not affect generalization of methamphetamine to the methamphetamine cue (Figure 5, bottom panel). Pretreatment with Albu-CocH did not affect the rate of responding under either test (cocaine or methamphetamine) condition (Data not shown).

Antibodies to Albu-CocH

The monkeys trained to self-administer cocaine and also tested on the reinstatement procedure received a total of 4 injections of Albu-CocH. Blood was taken for determination of Albu-CocH antibodies after the first, second and fourth Albu-CocH injections. Following the first 2 injections, antibody levels were below the level of detection (titer level of 20). Following the fourth injection, however, antibodies were detected. In 2 monkeys the antibody titer was slightly elevated over the limit of detection (21 and 43 units). For the third monkey, antibody levels were clearly elevated (643 units). The concentration of Albu-CocH in the serum for this monkey one week following the Albu-CocH injection was also reduced (27 ng/ml vs. 55 and 53 ng/ml for the other 2 monkeys).

Discussion

The results of these studies show that pretreatment with a modified form of BChE is effective in antagonizing the behavioral effects of cocaine in non-human primates. This work replicates and extends previous work with this compound in rats showing that Albu-CocH could antagonize cocaine toxicity and cocaine's behavioral effects (Brimijoin et al., 2008, Carroll et al., 2011). Here, in monkeys self-administering cocaine (30 μg/kg/injection), pretreatment with 5 mg/kg Albu-CocH markedly and significantly reduced both rate of responding for cocaine and the number of injections taken during daily sessions. Similarly, Albu-CocH was effective in blocking the reinstatement of extinguished drug-seeking behavior produced by a priming injection of either 0.3 or 0.1 mg/kg cocaine. Although more variable and outside statistical significance, Albu-CocH had qualitatively similar effects when the dose available for self-administration was reduced to 10 μg/kg. Finally, Albu-CocH was also able to antagonize the generalization of cocaine to a methamphetamine discriminative stimulus. This effect was specific to cocaine as evident in the inability of Albu-CocH to similarly antagonize the discriminative stimulus effects of methamphetamine.

During testing with 30 μg/kg cocaine self-administration, the effects of Albu-CocH were still evident 3 days after administration, although at reduced effectiveness. In reinstatement and discrimination testing, however, the effects of Albu-CocH were not evident 48 hours following treatment. The half-life of Albu-CocH was shown to be around 56 hours and effects on cocaine metabolism were still evident 72 hours after Albu-CocH administration, so it might have been expected that some effect on these procedures would have been evident at 72 hours. A number of factors could contribute to this failure to see a reduction in reinstatement or cocaine generalization at 72 hrs. During self-administration, smaller doses of cocaine are given over an extended period of time whereas, in the other procedures, a relatively larger dose of cocaine was administered as a bolus injection. Thus, it may be easier for Albu-CocH to metabolize the smaller amounts of cocaine under the self-administration conditions than under the other conditions. When Albu-CocH metabolizes cocaine under self-administration, its effect is to institute extinction conditions. Behavioral recovery from extinction may also prolong the effects of Albu-CocH treatment on self-administration. That is, the monkeys must actively self-administer cocaine in an amount sufficient to detect cocaine in the presence of the diminishing effects of Albu-CocH. In contrast, in the other studies, a bolus injection of cocaine is given by the experimenter.

The effects of Albu-CocH were almost certainly related to its effects on the metabolism of cocaine. The administration of Albu-CocH decreased the amount of cocaine in plasma for at least 72 hours. The fact that ecgonine methyl ester levels were also increased for at least 72 hours following Albu-CocH suggests that Albu-CocH was metabolizing cocaine similarly to native BChE (Jones, 1984). This time course for cocaine metabolism is similar to that seen for the self-administration experiment where the monkeys were reinforced with 30 μg/kg injections of cocaine. Further, Albu-CocH had no effect on response rate in the discrimination study, suggesting that it does not produce a non-specific effect on operant responding independent of the presence of cocaine in the blood.

The observation of relatively high levels of Albu-CocH antibodies in one monkey following its fourth injection indicates that Albu-CocH might lose some of its effectiveness over multiple injections. While the behavioral results for that one particular monkey did not appear to be different from the other 2 monkeys tested, the observation of antibodies suggests that some of the Albu-CocH may be bound by antibodies which, in turn, may decrease its ability to metabolize cocaine. The formation of antibodies following repeated dosing in monkeys is not surprising because Albu-CocH is a human protein being

administered to monkeys. Further work will be needed to evaluate the stimulation of antibody production in monkeys and its implications for the effectiveness of Albu-CocH in metabolizing cocaine in humans.

Previous work has shown the Albu-CocH can decrease the toxic effect of cocaine in rodents (Brimijoin et al., 2008) similarly to other cocaine esterases (Cooper et al., 2006, Collins et al., 2009, Lynch et al., 1997, Mattes et al., 1997, Wood et al., 2010). Although the toxic effects of cocaine were not studied in the present experiments, it seems likely that similar effects would be observed in non-human primates and humans. Therefore, Albu-CocH may well be useful in the treatment of acute cocaine toxicity. Cocaine continues to show up in both emergency room mentions and medical examiner reports in the DAWN surveys. As such, a drug that could counteract the toxic effects of cocaine may be a useful adjunct to emergency room treatment for patients abusing cocaine. An advantage of using a drug that metabolizes cocaine is that it will be specific for cocaine and should have minimal side effects. However, it would be necessary to confirm that a patient is using cocaine, as Albu-CocH would not be effective against toxicity produced by other drugs of abuse, such as the amphetamines, that might produce a similar spectrum of toxic effects.

In view of the lack of effective medications, the present results suggest that Albu-CocH might be useful in the management of cocaine abuse and addiction. A person with a sufficient blood level of Albu-CocH would be expected to experience a reduced subjective effect of cocaine. In the context of the current study, this translated to less cocaine self-administration, reduced reinstatement of extinguished cocaine-seeking behavior and the failure of cocaine to generalize to the discriminative stimulus produced by psychomotor stimulants. In a treatment setting, reduced subjective effects of cocaine may translate into a reduction in relapse liability. Importantly, the present results also indicate that continued effectiveness against relapse would depend on the continuous presence of Albu-CocH in the blood. This would necessitate treatment throughout periods of vulnerability, and further studies would be needed to evaluate the effects of such regimens. Further increases in half-life of CocH would also increase its usefulness as a drug abuse treatment. Gao and Brimijoin (2009) recently use a viral gene transfer approach with another quadruple mutant of human BChE to increase the level of the hydrolase in rat blood for up to 2 months. Further, Gao et al. (2010) have suggested that even the shorter duration forms of a cocaine hydrolase may improve the effectiveness of cocaine antibody therapy in the treatment of cocaine abuse by facilitating the removal of cocaine from the blood following multiple cocaine dosings that may occur during cocaine binges.

In conclusion, pretreatment of squirrel monkeys with Albu-CocH was able to reduce the amount of cocaine in the plasma following a cocaine bolus treatment. Pretreatment with Albu-CocH was also able to reduce cocaine self-administration in monkeys that had been trained to self-administer 30 μg/kg cocaine injections. Albu-CocH was able to block cocaine-induced reinstatement of cocaine-seeking behavior produced by 2 different doses of cocaine. Finally, Albu-CocH was able to antagonize the discriminative effects of cocaine in squirrel monkeys. The finding that Albu-CocH was able to antagonize the behavioral effects of cocaine in these different models of cocaine's behavioral effects suggests that it also might be valuable for managing cocaine abuse and addiction.

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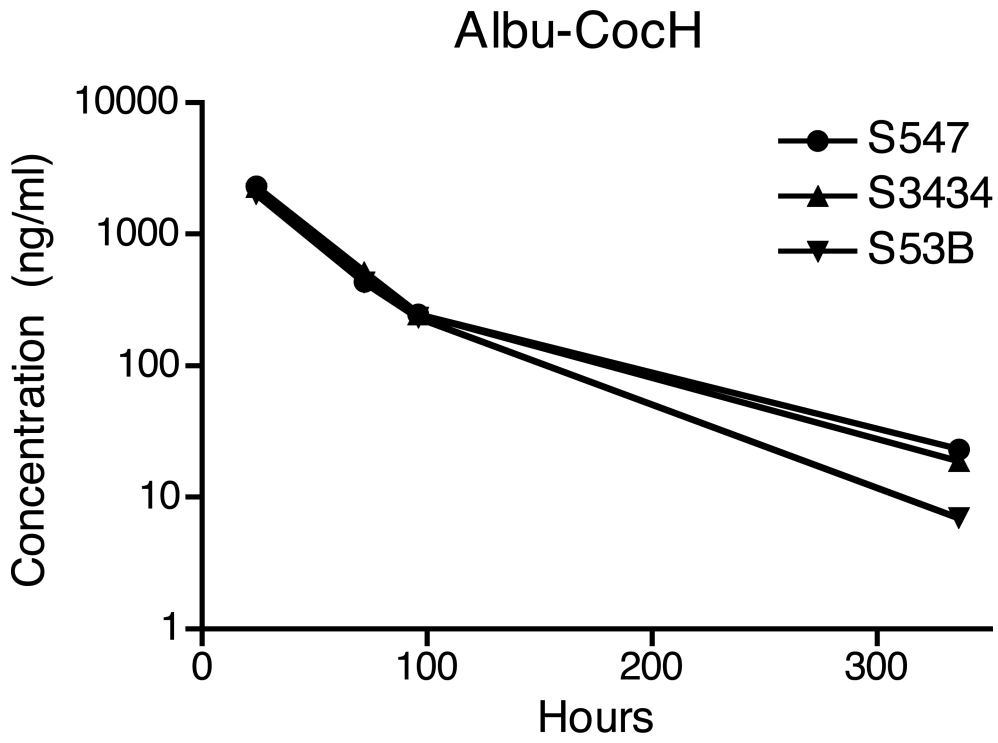

Figure 1.
Serum levels (ng/ml) of Albu-CocH for 3 individual monkeys at 24, 72, 96 and 336 hours following an i.m. injection of 5 mg/kg Albu-CocH.

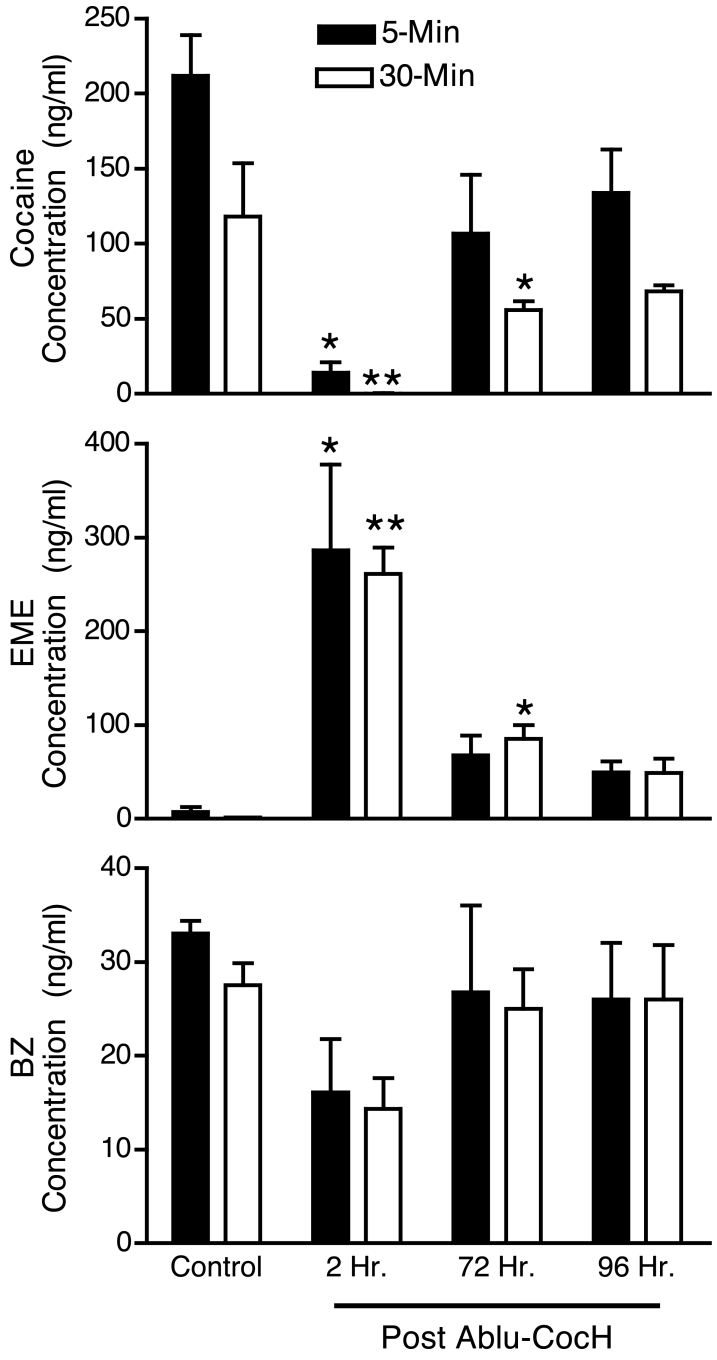

Figure 2.

Plasma concentrations of cocaine (top panel) and the cocaine metabolites ecgonine methyl ester (EME, middle panel) and benzoylecgonine (BZ, bottom panel) at 5 (top panel) and 30 min (bottom panel) following the cocaine injection. Two control squirrel monkeys (left-hand bars) received Albu-CocH vehicle i.m. and then were given 1 mg/kg cocaine i.v. 2 hours later. Three other squirrel monkeys were given 5 mg/kg Albu-CocH i.m. and then given 1 mg/kg cocaine i.v. 2, 24 and 72 hours later. *$p < 0.05$ from control.

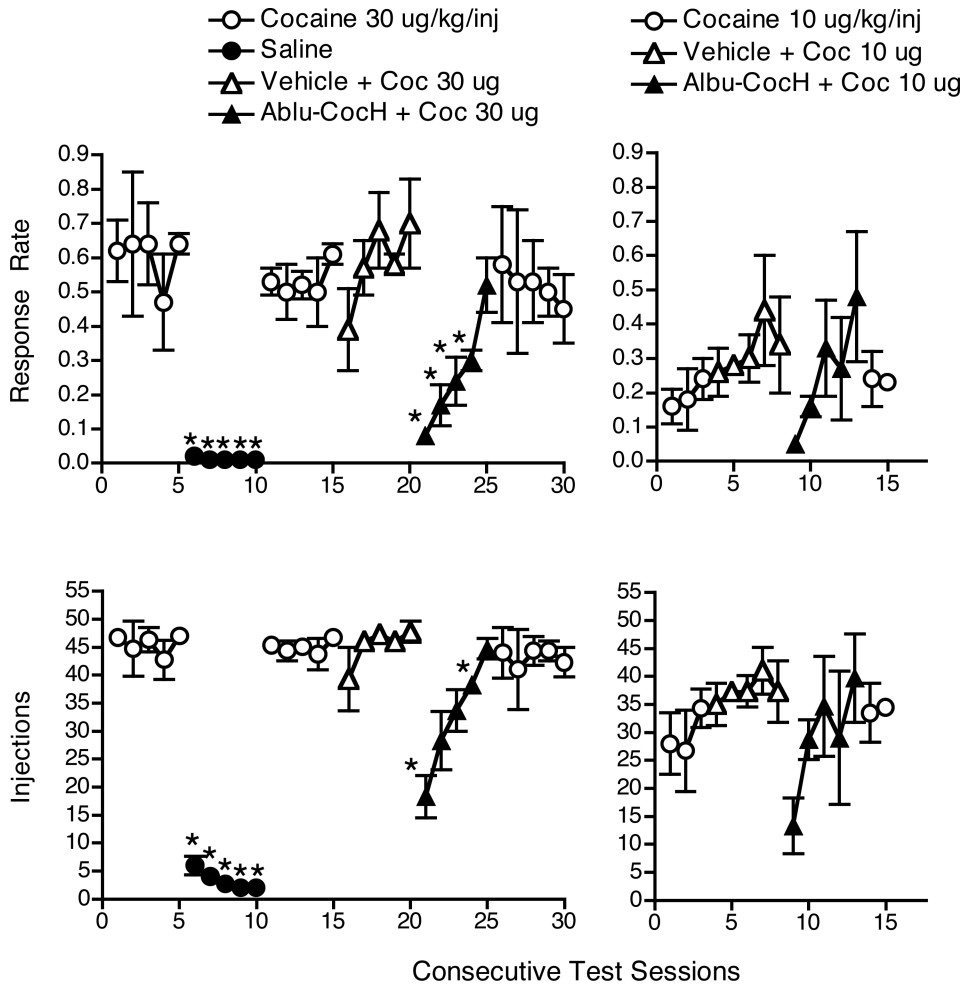

Figure 3.

Response rates (top panels) and injections (bottom panels) on consecutive self-administration sessions (open circles) for 3 monkeys trained with 30 μg/kg/injection cocaine (left-hand panels) or 10 μg/kg/cocaine (right-hand panels). Substituting saline for cocaine (closed circles) led to an immediate reduction in responding. Pretreatment with Albu-CocH vehicle 2 hrs before the first session of 5 consecutive sessions did not affect responding or injections. However, pretreatment with 5 mg/kg Albu-CocH 2 hrs before the first session of 5 consecutive sessions led to an immediate reduction of both responding and injections that recovered over the 5 days. *$p < 0.05$ from the average of the last 3 days of the previous baseline.

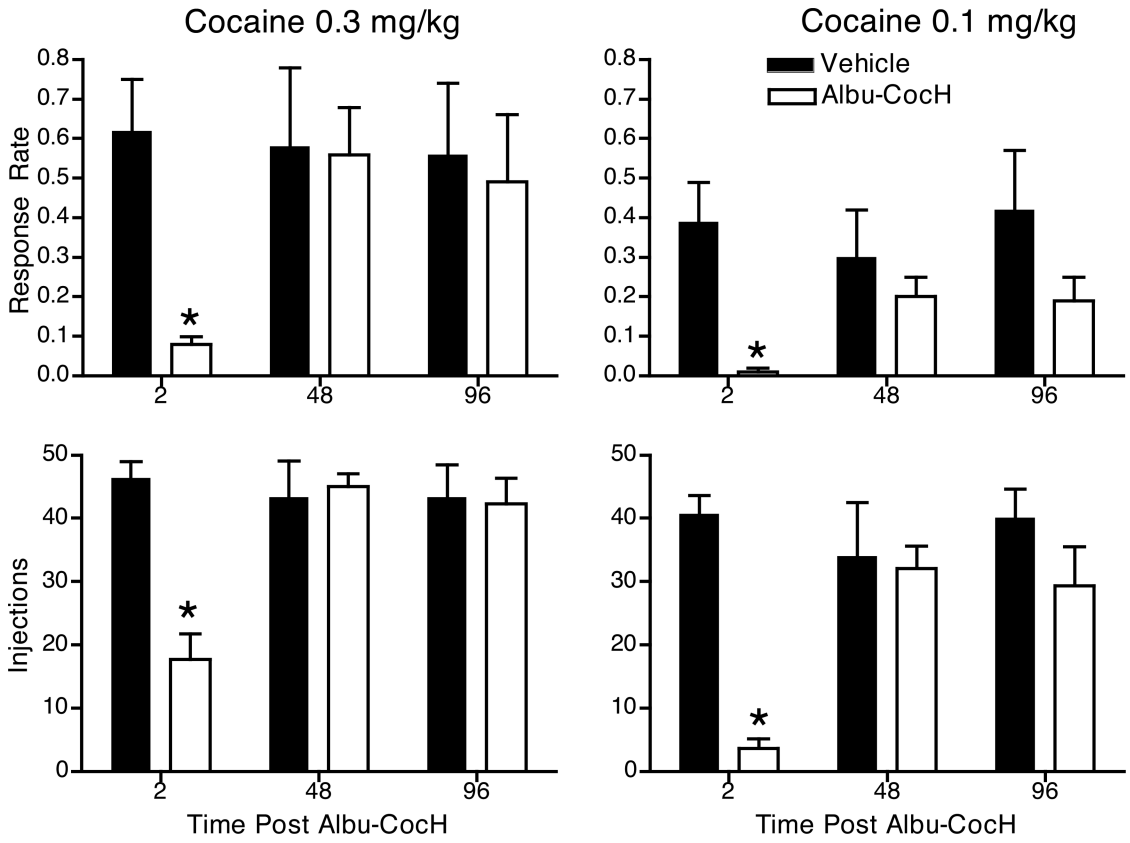

Figure 4.

Response rate (top panels) and injections (bottom panels) following a 0.3 mg/kg (left-hand panels) or 0.1 mg/kg (right-hand panels) priming injection of cocaine in 3 monkeys that had previously undergone extinction training from 30 μg/kg/injection cocaine self-administration. Filled bars are reinstatement sessions 2, 48 and 96 hours following an injection of Albu-CocH vehicle. Open bars are reinstatement sessions 2, 48 and 96 hours following an injection of 5 mg/kg Albu-CocH. *$p < 0.05$ from vehicle treatment at that time point. Albu-CocH blocked reinstatement when given 2 hours before cocaine, but not 48 or 96 hours prior to cocaine.

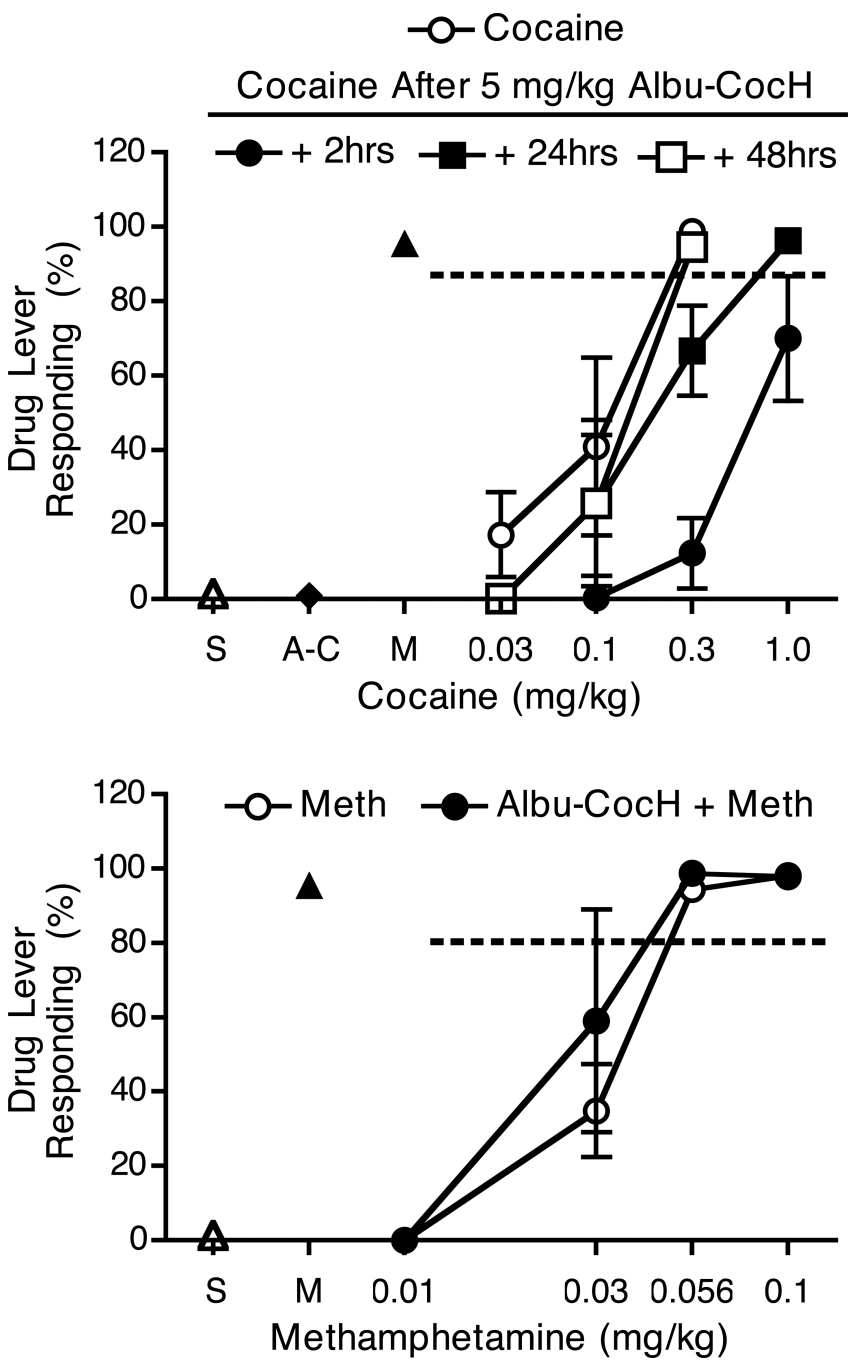

Figure 5.

Drug lever responding in 4 monkeys trained to discriminate 0.56 mg/kg methamphetamine from saline. The top panel shows discrimination tests for cocaine alone and for cocaine 2, 24 and 48 hours following treatment with 5 mg/kg Albu-CocH. The point above ‘S” is for a saline test, the point above A-C is for a 5 mg/kg Albu-CocH test in the absene of cocaine and the point above M is for a test of the methamphetamine training dose. Albu-CocH shifted the cocaine dose-effect function to the right when given 2 hours before the test. All cocaine substitution points below 50% were significantly different from the methamphetamine control. The bottom panel shows discrimination tests for

methamphetamine alone and 2 hrs following treatment with 5 mg/kg Albu-CocH. Albu-CocH did not shift the methamphetamine dose-effect function.