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Effects of a long-acting mutant bacterial cocaine esterase on acute cocaine toxicity in rats

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Abstract

Background—A longer acting, double mutant bacterial cocaine esterase (CocE T172R/G173Q; DM CocE) has been shown to protect mice from cocaine-induced lethality, inhibit the reinforcing effects of cocaine in rats, and reverse cocaine's cardiovascular effects in rhesus monkeys. The current studies evaluated the effectiveness of DM CocE to protect against, and reverse cocaine's cardiovascular, convulsant, and lethal effects in male and female rats.

Methods—Pretreatment studies were used to determine the effectiveness and in vivo duration of action for DM CocE to protect rats against the occurrence of cardiovascular changes, convulsion and lethality associated with acute cocaine toxicity. Posttreatment studies were used to evaluate the capacity of DM CocE to rescue rats from the cardiovascular and lethal effects of large doses of cocaine. In addition, male and female rats were studied to determine if there were any potential effects of sex on the capacity of DM CocE to protect against, or reverse acute cocaine toxicity in rats.

Results—Pretreatment with DM CocE dose-dependently protected rats against cocaine-induced cardiovascular changes, convulsion and lethality, with higher doses active for up to 4 hrs, and shifting cocaine-induced lethality at least 10-fold to the right. In addition to dose-dependently recovering rats from an otherwise lethal dose of cocaine, post-treatment with DM CocE also reversed the cardiovascular effects of cocaine. There were no sex-related differences in the effectiveness of DM CocE to protect against, or reverse acute cocaine toxicity.

Conclusions—Together, these results support the development of DM CocE for the treatment of acute cocaine toxicity.

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Contributors

Author GTC designed the study, analyzed the data, and managed the literature search. Authors GTC, MEZ, ARC, CS, DN and JN performed research and analyzed data. Author GTC wrote the first draft of the manuscript and all authors revised and approved the final manuscript.

Conflict of interest

RKS, MCK, DN, and JHW are listed as inventors on the patent for DM CocE. RKS and JHW have received consulting funds from Reckitt Benckiser Pharmaceuticals concerning cocaine esterase. The University of Michigan, Columbia University, and the University of Kentucky have licensed the potential development and use of cocaine esterase and mutants to Reckitt Benckiser Pharmaceuticals for acute cocaine toxicity.

Keywords

Cocaine; Cocaine Esterase; Acute Cocaine Toxicity; Convulsion; Lethality; Cardiovascular; Rat

1. Introduction

Cocaine abuse remains a significant public health problem with an estimated 20 million individuals using cocaine within the past year worldwide. Although cocaine use has been increasing in certain European countries, approximately half of all annual cocaine use occurs in North and South America (United Nations Office on Drugs and Crime, 2010). In the United States alone, 1.1 million individuals were identified as dependent on, or abusing cocaine during the 2009 calendar year (Substance Abuse and Mental Health Services Administration, 2010b). Cocaine also accounts for the majority of illicit drug-related emergency room (ER) visits in the U.S., with over 550,000 annual cases according to recent estimates (Substance Abuse and Mental Health Services Administration, 2010a). In humans, acute cocaine intoxication has a variety of symptoms, including agitation, anxiety, delirium, hypothermia, and convulsion (Glauser and Queen, 2007; Olson et al., 1993). However, the most commonly reported reason for ER admission is cocaine-associated chest pain resulting from the hypertensive and tachycardic effects of cocaine (Brody et al., 1990; Mittleman et al., 1999). Although current guidelines suggest that acute cocaine toxicity be symptomatically treated with intravenous benzodiazepines (McCord et al., 2008), there are no approved therapeutics for the treatment of acute cocaine toxicity.

Recently, a significant effort has been made towards developing protein-based therapeutics, including cocaine-specific antibodies (Haney et al., 2010; Haney and Kosten, 2004; Martell et al., 2005), and cocaine-specific enzymes or catalytic antibodies (Landry et al., 1993; Turner et al., 2002; Zheng et al., 2008), that are aimed at reducing the reinforcing and toxic effects of cocaine by limiting the amount of cocaine that is able to reach its central site(s) of action. Although cocaine is naturally metabolized by butyrylcholinesterase (BChE) to the inactive metabolites ecgonine methyl ester and benzoic acid in both primates and rodents, this hydrolysis occurs relatively slowly ($K_{cat}/K_m = 9.1 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$; Sun et al., 2002), resulting in an elimination half-life for cocaine of approximately 26 minutes in rats (Carmona et al., 2005), and approximately 45 minutes in non-human and human primates (Mello et al., 2002; Mendelson et al., 1999). Cocaine-specific enzymes enhance the metabolism of cocaine, and effectively shorten cocaine's duration of action. Although the administration of exogenous BChE has been shown to produce modest reductions in the toxic effects of cocaine (Lynch et al., 1997), site-directed mutagenesis studies have identified mutant BChEs that are approximately 450- to 2000-times more efficient at hydrolyzing cocaine than native BChE (Pan et al., 2005; Zheng et al., 2008). When administered to rats and mice, these mutant BChEs are not only capable of preventing the lethal effects of cocaine, but also blunting the cardiovascular and reinforcing effects of cocaine (Brimijoin et al., 2008; Carroll et al., 2010; Xue et al., 2010; Zheng et al., 2008).

In addition to mutant BChEs, a bacterial cocaine esterase (CocE) isolated from *Rhodococcus* sp. strain MB1 growing in the rhizosphere soil beneath coca plants in South America (Bresler et al., 2000) has also been extensively evaluated as an alternative approach to enhancing the metabolism of cocaine in mice, rats, and rhesus monkeys. The wild-type (wt) form of CocE is capable of not only preventing the convulsant and lethal effects of cocaine in mice and rats (Cooper et al., 2006; Ko et al., 2007; Ko et al., 2009), but also reversing the cardiovascular and seizurogenic effects of cocaine in rats (Jutkiewicz et al., 2009; Wood et al., 2010). However, the relatively short *in vivo* duration of wt CocE's actions ($t_{1/2} \sim 15$ minutes; Cooper et al., 2006) has limited its development as a treatment for cocaine abuse or

toxicity. Recently, site-directed mutagenesis studies have identified a mutant form of CocE (T172R/G173Q; double mutant CocE; DM CocE), with an *in vivo* duration of action of approximately 4.5 hrs in mice (Gao et al., 2009; Narasimhan et al., 2010). DM CocE has been shown to dose-dependently reduce the reinforcing effects of cocaine in rats (Collins et al., 2009), and ameliorate the cardiovascular effects of cocaine in rhesus monkeys (Collins et al., 2011), suggesting that DM CocE could provide a valuable therapeutic option for the treatment of acute cocaine toxicity and abuse. Despite these promising findings, the capacity of DM CocE to protect against, and reverse acute cocaine toxicity in rats has yet to be fully characterized. In addition, to date there have been no studies in which potential for sex-related differences in the effectiveness of highly efficient cocaine hydrolyzing enzymes has been systematically evaluated.

Therefore, the goals of the current studies were to 1) characterize the capacity of DM CocE to protect against cocaine-induced convulsion and lethality, 2) to determine the duration of DM CocE's protective effects against an LD₁₀₀ dose of cocaine, 3) to assess the protective effects of DM CocE against the cardiovascular effects of IV cocaine, 4) to evaluate the capacity of DM CocE to dose-dependently recover rats from the lethal effects of an LD₁₀₀ dose of cocaine when administered 1 minute after the onset of convulsion, 5) to evaluate the capacity of DM CocE to ameliorate cocaine-induced changes in mean arterial pressure (MAP) and heart rate (HR) when administered immediately after the onset of convulsion produced by an LD₁₀₀ dose of cocaine, as well as when administered 10 minutes after IV cocaine, and 6) to determine if there were any sex-related differences in the effects of DM CocE.

2. Methods

2.1 Subjects

Male (250–275g) and female (200–225g) Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN), and individually housed in a temperature-(21–23 °C), and humidity-controlled environment, on a 12-hr dark/light cycle with lights on at 7:00 AM. All animals had free access to standard Purina rodent chow and tap water, with the exception of during experimentation. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals and performed in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.

2.2 Surgical preparation

Prior to experimentation, all rats were prepared with an indwelling jugular catheter under ketamine:xylazine (90:10 mg/kg; IP) anesthesia. The areas above the scapula and jugular vein were shaved and cleaned with alternating betadine/alcohol scrubs prior to small incisions being made to allow for catheter placement. Upon implantation, the catheter was passed under the skin and exited through the incision made between the scapula. For cardiovascular studies, additional incisions were made above the femoral artery and on the back to allow for a telemetric probe (model PA-C40; Data Sciences International, St Paul, MN) capable of collecting mean arterial pressure (MAP), and heart rate (HR) to be implanted. Upon the subcutaneous placement of the probe, the pressure transducer was passed under the skin and inserted into the femoral artery. All incisions were closed with 5-0 Ethilon® suture, and rats were allowed 5–7 days to recover from surgery. Catheters were flushed daily with heparinized saline (100 U/ml) to ensure catheter patency.

2.3 Procedures

2.3.1 Observational assays—Acute cocaine toxicity was defined as the occurrence of cocaine-induced convulsion (loss of the righting reflex followed by clonic leg movements) and lethality (cessation of any visible movement and respiration). On the day of testing, rats were transferred from their home cage to an observation chamber (48 cm × 23 cm × 20 cm clear rodent cage with cob bedding), and provided a 30-minute habituation period. DM CocE or vehicle (phosphate-buffered saline; PBS) were administered IV, and cocaine was administered IP at the specified times, and rats were continuously observed for 60 minutes thereafter with the time to convulsion and/or lethality recorded. During recovery studies catheters were attached to Tygon® tubing filled with PBS which exited the chamber through a stainless steel spring tether to allow for the IV administration of DM CocE or PBS while the rats were convulsing, or without disturbing the rats (cardiovascular studies; below).

2.3.2 Cardiovascular assays—On test days, rats were transferred to observation chambers and attached to tethers as described above, with the exception that observation chambers were placed on receivers (Data Sciences International, St. Paul, MN) to allow for the collection of MAP and HR measures using Dataquest A.R.T. 3.01 software. Although cardiovascular measures were only collected for the 30 minutes immediately before and after the administration of cocaine and PBS or DM CocE, the rats were observed for 60 minutes after cocaine administration with the occurrence of convulsion and/or lethality recorded. With respect to cardiovascular measures, lethality was defined as a MAP below 10 mmHg.

2.3.3 Dose selection—The doses of cocaine that were used to evaluate the capacity of DM CocE to protect against, or reverse cocaine-induced convulsion and lethality were based on previous reports from our lab in which the 180.0 mg/kg IP dose of cocaine has been identified as the LD₁₀₀ dose for cocaine in rats (Collins et al., 2009; Cooper et al., 2006; Jutkiewicz et al., 2009; Wood et al., 2010). For protection studies (2.4.1), the maximal dose of cocaine that was administered was based on the solubility limit of cocaine given as a 1ml/kg injection. The doses of cocaine that were used to evaluate the capacity of DM CocE to protect against, or reverse cocaine-induced cardiovascular effects were based on previous studies in which the cardiovascular effects of IP (Wood et al., 2010), or IV (Brimijoin et al., 2008; Chen et al., 1995; Kiritsy-Roy et al., 1990; Mactutus et al., 2000; Pitts et al., 1987; Tella et al., 1992b) cocaine have been evaluated in conscious rats. The doses of DM CocE that were evaluated in the current study were based on previous work from our lab in which the capacities of wt CocE and DM CocE to protect against, or reverse acute cocaine toxicity in rats was studied (Collins et al., 2009; Cooper et al., 2006; Jutkiewicz et al., 2009; Wood et al., 2010).

2.4 Protection studies

2.4.1 Protective effects of DM CocE against cocaine-induced convulsion and lethality—To determine the capacity of DM CocE to protect against acute cocaine toxicity, rats (5 males and 5 females per dose combination) were pretreated with one of three doses of DM CocE (0.32, 1.0, or 3.2 mg/kg; IV) or PBS 1 minute prior to the IP administration of one of five doses of cocaine. PBS-treated rats were challenged with 0.0, 32.0, 56.0, 100.0, or 180.0 mg/kg; IP cocaine, whereas DM CocE-treated rats were challenged with 0.0, 180.0, 320.0, 560.0, or 1000.0 mg/kg; IP cocaine.

2.4.2 *In vivo* duration of action for DM CocE against cocaine-induced convulsion and lethality—To determine the duration of DM CocE's protective effects against acute cocaine toxicity, rats (5 males and 5 females per dose and time combination) were pretreated with one of four doses of DM CocE (0.32, 1.0, 3.2, or 10.0 mg/kg; IV) or

PBS at one of five times (1, 60, 120, 240, and 360 minutes) prior to 180.0 mg/kg; IP cocaine.

2.4.3 Protective effects of DM CocE against cocaine-induced cardiovascular changes—In order to evaluate the capacity of DM CocE to protect against the cocaine-induced cardiovascular changes, two groups of 6 male rats were pretreated with either PBS or 0.32 mg/kg; IV DM CocE, 1 minute prior to 5.6 mg/kg; IV cocaine.

2.5 Recovery studies

2.5.1 Capacity of DM CocE to recover rats from the toxic effects of 180.0 mg/kg; IP cocaine—In order to determine the capacity of DM CocE to rescue rats from the convulsant and lethal effects of cocaine, rats (5 males and 5 females per dose combination) were pretreated with an LD₁₀₀ dose of 180.0 mg/kg; IP cocaine, with one of three doses of DM CocE (0.32, 1.0, or 3.2 mg/kg; IV) or PBS administered 1 minute after the onset of convulsion.

2.5.2 Capacity of DM CocE to recover rats from the cardiovascular effects of 180.0 mg/kg; IP cocaine—Two separate groups of 10 male rats were used to assess the effectiveness of DM CocE to ameliorate the cardiovascular effects of 180.0 mg/kg; IP cocaine. PBS or 3.0 mg/kg DM CocE was administered immediately after the onset of convulsion to increase the probability of survival during the collection of MAP and HR measures.

2.5.3 Capacity of DM CocE to recover rats from the cardiovascular effects of 5.6 mg/kg; IV cocaine—In order to evaluate the capacity of DM CocE to ameliorate the cardiovascular effects of IV cocaine, two groups of 6 male rats were pretreated with either PBS or 0.32 mg/kg DM CocE 10 minutes after the administration of 5.6 mg/kg; IV cocaine.

2.6 Drugs

(–)-Cocaine HCl was purchased from Mallinckrodt/Tyco (St. Louis, MO, USA), dissolved in physiologic saline, and administered either intravenously (IV) or intraperitoneally (IP) in a volume of 1 ml/kg. Cocaine esterase (T172R/G173Q CocE; DM CocE) was provided by Reckitt Benckiser Pharmaceuticals in PBS at a concentration of 5.0 mg/ml, administered IV on a mg/kg basis.

2.7 Data Analysis

Data from the convulsion/lethality studies are presented as the occurrence percentage of convulsion or lethality, with significant differences between the DM CocE and PBS determined by one-tailed Fisher's exact probability tests. One-tailed Fisher's exact probability tests were also used to determine if there were any significant differences in the effects of cocaine in the absence or presence of DM CocE between male and female rats. The cardiovascular effects of cocaine are presented as the mean change in MAP or HR $\pm$ the standard error of the mean (SEM) from baseline (1-minute period before first injection) for successive 1-minute blocks of time. Significant differences in the cardiovascular effects of IV cocaine in the presence or absence of DM CocE were evaluated by two-way ANOVA with post-hoc Bonferroni tests. Significant differences from baseline MAP or HR measures during tests in which DM CocE or PBS was administered immediately after 180.0 mg/kg; IP cocaine-induced convulsion were determined with one-way ANOVA with post-hoc Dunnett's tests.

3. Results

3.1 Protection studies

3.1.1 Protective effects of DM CocE against cocaine-induced convulsion and lethality—As shown in Figure 1, cocaine induced dose-dependent increases in convulsion and lethality, with an LD₁₀₀ of 180.0 mg/kg IP in PBS-treated rats. Pretreatment with DM CocE (0.32, 1.0, or 3.2 mg/kg; IV) 1 minute prior to cocaine challenge (180.0, 320.0, 560.0, or 1000.0 mg/kg IP) produced a dose-dependent rightward shift in the dose-response curves for cocaine-induced convulsion and lethality. When administered prior to an LD₁₀₀ dose of 180.0 mg/kg IP cocaine, doses of 0.32 and 1.0 or 3.2 mg/kg DM CocE resulted in lethality rates of 20% and 0%, respectively. Although a 3-fold shift in the ED_{100conv} and LD₁₀₀ for cocaine was observed with 0.32 mg/kg DM CocE, ED_{100conv} or LD₁₀₀ values could not be obtained when rats were pretreated with either 1.0 or 3.2 mg/kg DM CocE prior to challenge doses as large as 1000.0 mg/kg; IP cocaine. These higher doses of DM CocE did, however, produce greater than 10-fold rightward shifts in the ED_{50conv} and LD₅₀ values for cocaine. As shown in Table 1, PBS-treated male and female rats did not differ in their sensitivity to cocaine-induced convulsion or lethality, and likewise, there were no significant effects of sex on the capacity of DM CocE to protect against cocaine-induced convulsion or lethality.

In addition to the dose-dependent increase in the percent of PBS-treated rats that displayed convulsion and lethality, a dose-dependent decrease in the time to convulsion [$F(2,24) = 14.16$; $p < 0.0001$], and lethality [$F(2,20) = 9.24$; $p < 0.05$] was also observed following increasing dose of cocaine (data not shown). Despite the dose-dependent decreases in the percentage of convulsion and lethality incidence (Figure 1), DM CocE did not systematically alter the time to convulsion or lethality following an LD₁₀₀ dose of 180.0 mg/kg IP as compared to PBS-treated rats, which was likely due to the small proportion of DM CocE-treated rats that convulsed or died (2 out of 30).

3.1.2 *In vivo* duration of action for DM CocE against cocaine-induced convulsion and lethality—In order to assess the *in vivo* duration of action for DM CocE, rats were pretreated with PBS or DM CocE at one of five times (1, 60, 120, 240, and 360 minutes) prior to a dose of 180.0 mg/kg; IP cocaine. As shown in Figure 2, this dose of cocaine induced convulsion followed by lethality in 100% of the PBS-treated rats, regardless of the pretreatment time. Although the lowest dose of DM CocE (0.32 mg/kg; IV) failed to protect more than 50% of the rats when administered 1 minute before 180.0 mg/kg; IP cocaine, doses of 1.0, 3.2, and 10.0 mg/kg DM CocE were fully effective at preventing the occurrence of convulsion and lethality. Moreover, the duration of DM CocE's protective effects (pretreatment time at which ED_{50conv} and LD₅₀ values for cocaine were observed) was dose-dependent, with doses of 1.0, 3.2, and 10.0 mg/kg DM CocE lasting approximately 45 minutes, 100 minutes, and 240 minutes, respectively. As was observed when DM CocE was administered prior to increasing doses of cocaine, there was no significant effect of sex on the duration of DM CocE's protective effects against cocaine-induced convulsion and lethality (Table 2).

3.1.3 Protective effects of DM CocE against cocaine-induced cardiovascular changes—In PBS-treated rats, 5.6 mg/kg; IV cocaine produced a rapid onset of convulsion (14.8 ± 1.2 seconds) in 83% of rats, with recovery observed in 100% of rats (i.e., 0% lethality) with an average latency of 126.2 ± 26.2 seconds. Administration of 0.32 mg/kg DM CocE 1 minute prior to 5.6 mg/kg; IV cocaine completely inhibited the occurrence of convulsion. In addition, when compared to the increases in MAP and decreases in HR that were observed in PBS-treated rats, pretreatment with 0.32 mg/kg DM CocE significantly

reduced the MAP increasing ($F[1,200]=214.1$; $p<0.001$), and HR decreasing ($F[1,200]=141.9$; $p<0.001$) effects of cocaine (Figure 3).

3.2 Recovery studies

3.2.1 Capacity of DM CocE to recover rats from the toxic effects of 180.0 mg/kg; IP cocaine—In addition to protecting against the cardiovascular, convulsant, and lethal effects of cocaine, DM CocE was also capable of dose-dependently recovering rats from an otherwise LD_{100} dose of 180.0 mg/kg; IP cocaine when administered 1 minute after the onset of convulsion (Figure 4). Although 0.32 mg/kg DM CocE was only capable of recovering 20% of rats (i.e., 80% lethality), pretreatment with doses of 1.0 mg/kg and 3.2 mg/kg DM CocE rescued a statistically significant percentage of the rats with survival rates of 80% and 90% (i.e., 20% and 10% lethality), respectively. The time to convulsion did not differ among the four treatment groups with an average latency to convulsion of 3–5 minutes. Although larger doses of DM CocE tended to recover rats from convulsion sooner (7.0 ± 5.2 , 3.4 ± 1.1 , and 2.1 ± 0.7 minutes with 0.32, 1.0, and 3.2 mg/kg DM CocE, respectively), these differences failed to reach significance. In addition, there were no significant effects of sex on either the convulsant or lethal effects of cocaine in PBS-treated rats or the capacity of DM CocE to rescue rats from the lethal effects of 180.0 mg/kg; IP cocaine (Table 3).

3.2.2 Capacity of DM CocE to recover rats from the cardiovascular effects of 180.0 mg/kg; IP cocaine—Similar effects of 180.0 mg/kg; IP cocaine were also observed in telemetered rats, with convulsion observed in 100% of rats, with an average latency to convulsion of 3.7 ± 0.7 , and 3.7 ± 0.3 minutes in rats that later received PBS, and 3.0 mg/kg DM CocE, respectively. Although 100% lethality was observed in the PBS-treated group by minute 10, all DM CocE-treated rats survived the entire 60-minute period. At the time of convulsion, cocaine produced significant increases in MAP (PBS: $+42.7\pm5.5$; and DM CocE: $+39.9\pm4.3$ mmHg; $p<0.001$ for both), and significant decreases in HR (PBS: -49.6 ± 9.8 ; and DM CocE: -64.5 ± 7.6 bpm; $p<0.001$ for both) in both groups of rats (Figure 5). When administered immediately after the onset of convulsion, 3.0 mg/kg DM CocE not only prevented lethality in 100% of rats, but also reversed the increases in MAP and decreases in HR produced by an IP dose of 180.0 mg/kg cocaine. Unlike the precipitous decrease in MAP that accompanied death in PBS-treated rats, administration of 3.0 mg/kg DM CocE returned MAP to baseline-like levels within 5 minutes, where it remained at baseline levels for the remainder of the 20-minute period. Administration of 3.0 mg/kg DM CocE had similar effects on the cocaine-induced changes in HR, however, unlike with MAP, HR was no different than baseline at any point after DM CocE administration.

3.2.3 Capacity of DM CocE to recover rats from the cardiovascular effects of 5.6 mg/kg; IV cocaine—As was observed when rats were pretreated with PBS 1 minute before an IV dose of 5.6 mg/kg cocaine, convulsion occurred in 83% of rats that later received PBS or 0.32 mg/kg DM CocE. There were no differences in the latencies to convulsion onset (PBS: 22.6 ± 5.0 seconds, and DM CocE: 29.8 ± 6.1 seconds), or recovery from convulsion (PBS: 141.8 ± 25.5 seconds, and DM CocE: 96.6 ± 35.0 seconds) between the two groups, with 100% of rats recovering from convulsion prior to the administration of PBS or DM CocE. In addition, 5.6 mg/kg; IV cocaine also produced similar increases in MAP, and decreases in HR in both groups of rats during the 10-minute period prior to administration of PBS or DM CocE (Figure 6). Administration of 0.32 mg/kg DM CocE, 10 minutes after cocaine resulted in a rapid amelioration of the MAP increasing effects of cocaine ($F[1,300]=118.7$; $p<0.001$), with significant reductions in MAP observed within 7 minutes of DM CocE administration. Although DM CocE significantly attenuated the

hypertensive effects of cocaine, post-treatment with 0.32 mg/kg DM CocE failed to significantly alter the HR decreasing effects of 5.6 mg/kg; IV cocaine.

4. Discussion

In rodents, acute cocaine toxicity is marked by increases in blood pressure, decreases in heart rate, respiratory suppression, and tonic-clonic convulsions; the latter is associated with status epilepticus and thought to be the primary mechanism responsible for cocaine-induced lethality (Tella et al., 1992a; Tseng et al., 1992). The current studies demonstrated the capacity of DM CocE to prevent and reverse these markers of acute cocaine toxicity in male and female rats with five main findings. First, pretreatment with DM CocE produced a dose-dependent rightward shift in the capacity of cocaine to induce convulsion and lethality, with doses of 1.0 mg/kg DM CocE and larger producing at least a 10-fold shift in cocaine's convulsant and lethal effects. Second, the protective effects of DM CocE were long lasting, with a dose of 10.0 mg/kg DM CocE fully protecting 50% of rats when administered up to 4 hours before an otherwise LD₁₀₀ dose of 180.0 mg/kg; IP cocaine. Third, when administered 1 minute before cocaine, a low dose of 0.32 mg/kg DM CocE significantly reduced the convulsant, hypertensive, and bradycardic effects of a sub-lethal IV dose of 5.6 mg/kg cocaine. Fourth, DM CocE dose-dependently recovered male and female rats from acute cocaine toxicity when administered 1 minute after the onset of convulsion produced by an otherwise LD₁₀₀ dose of 180.0 mg/kg; IP cocaine. Fifth, DM CocE was capable of ameliorating the cardiovascular effects of cocaine when administered either immediately after the onset of 180.0 mg/kg; IP cocaine-induced convulsion, or 10 minutes after an IV dose of 5.6 mg/kg cocaine. These studies provide strong evidence that DM CocE is capable of not only preventing, but also reversing the cardiovascular, convulsant, and potentially lethal effects of cocaine in male and female rats, and when taken together they suggest that highly efficient cocaine-hydrolyzing enzymes, such as DM CocE, may provide a valuable therapeutic option for the treatment of acute cocaine toxicity in humans.

These studies confirm previous reports in which wt CocE has been shown to dose-dependently protect against, and antagonize the cardiovascular, convulsant, and lethal effects of an otherwise LD₁₀₀ dose of cocaine in mice and rats (Cooper et al., 2006; Jutkiewicz et al., 2009; Ko et al., 2007; Ko et al., 2009; Wood et al., 2010), and extend those in which the introduction of thermostabilizing mutations (T172R and G173Q; Gao et al., 2009; Narasimhan et al., 2010) have been shown to significantly improved the in vivo duration of CocE's action without altering its effectiveness to protect against and reverse acute cocaine toxicity in rodents. In addition to providing a thorough evaluation of the capacity of DM CocE to protect against, and reverse acute cocaine toxicity in rats, it is important to note that these are the first studies to characterize the effects of CocE, wt or DM, in both male and female rats. Although it has been reported that male rats are more sensitive to the lethal effects of IV cocaine than female rats (e.g., Morishima et al., 1993), the current studies did not find any systematic differences in the effects of IP cocaine, or DM CocE between male and female rats. This is perhaps best illustrated by the finding that a dose of 180.0 mg/kg; IP cocaine was lethal in 100% of PBS-treated male (n=35) and female (n=35) rats, whereas lethality was not observed in any of the rats, male or female, that were treated with DM CocE (1.0 mg/kg or greater) 1 minute prior to the administration of 180.0 (n=25 males/25 females), or 320.0 mg/kg; IP cocaine (n=10 males/10 females). When taken together with the findings of a recent study in which DM CocE effectively ameliorated the cardiovascular effects of cocaine in male and female rhesus monkeys (Collins et al., 2011), these studies suggest that sex does not significantly affect the capacity of DM CocE to protect against or reverse acute cocaine toxicity.

In addition to demonstrating the power of DM CocE to protect against the convulsant and lethal effects of cocaine, the current studies also evaluated the capacity of DM CocE to affect the cardiovascular effects of 5.6 mg/kg; IV cocaine. Consistent with previous reports (Chen et al., 1995; Kiritsy-Roy et al., 1990; Mactutus et al., 2000; Pitts et al., 1987; Tella et al., 1992b), this relatively low dose of 5.6 mg/kg; IV cocaine increased MAP, decreased HR, and induced convulsion in approximately 80% of rats. Pretreatment with a low dose of DM CocE (0.32 mg/kg) not only effectively blunted the cocaine-induced changes in MAP and HR, but also completely prevented the onset of convulsion; effects that are similar to those observed with a 3 mg/kg dose of the mutant BChE, Albu-CocH (Brimijoin et al., 2008). Curiously, although the effects of DM CocE on MAP were apparent immediately after cocaine administration, a significant reversal of cocaine's bradycardic effects was not observed until 12 minutes after cocaine administration. A similar distinction was observed when 0.32 mg/kg DM CocE was administered 10 minutes after 5.6 mg/kg; IV cocaine, with a significant reduction in MAP observed shortly after DM CocE administration, and no change in the rate at which the HR measures returned to baseline. Although it is unclear if higher doses of DM CocE would have reversed the bradycardic effects of cocaine, it is important to note that DM CocE significantly attenuated the HR decreasing effects of cocaine in rats when administered either shortly before 5.6 mg/kg; IV cocaine, or shortly after 180.0 mg/kg; IP cocaine. In addition, when evaluated in the rhesus monkey, a species with a much more human-like cardiovascular response to cocaine (Foltin and Fischman, 1991; Javaid et al., 1978), posttreatment with DM CocE 10 minutes after cocaine resulted in a dose-dependent reversal of both the hypertensive and tachycardic effects of cocaine (Collins et al., 2011), suggesting that the inability of DM CocE to reverse cocaine's bradycardic effects in the present studies may have been due to the relatively short-lived nature of cocaine's cardiovascular effects in the rat. Alternatively, cocaine is also known to suppress the pacemaker function of the sinoatrial node and stimulate sympathetic activity (e.g., Przywara and Dambach, 1989; Trendelenburg, 1968), raising the possibility that the bolus administration 5.6 mg/kg cocaine into the jugular vein was sufficient to produce a suppression of sinoatrial node function that persisted even after cocaine was eliminated.

Although many factors contribute to the difficulty in establishing a direct correlation between cocaine blood levels and acute toxicity in humans, the need for therapeutics that are capable of rapidly antagonizing/reversing the effects of both small and large amounts of cocaine is highlighted by the wide range of cocaine blood levels (>100 ng/ml to well over 4000 ng/ml) that have been reported for emergency room patients and post-mortem overdose fatalities (Blaho et al., 2000; de Prost et al., 2010; Finkle and McCloskey, 1978; Karch et al., 1998; Koehler et al., 2005). In addition to demonstrating the power of DM CocE to rapidly eliminate large amounts of cocaine, such as those that might be observed with "body packers" or "drug mules," the current studies also evaluated DM CocE against smaller doses of cocaine that may more closely approximate cocaine levels associated with common patterns of cocaine use (e.g., intra-nasal, smoked, or IV cocaine). In PBS-treated rats, an IP dose of 180.0 mg/kg cocaine produced convulsion followed by lethality in 100% of the male and female rats tested (n=70). Pretreatment with DM CocE resulted in dose-dependent rightward shift in the convulsant and lethal effects of cocaine, with a dose of 3.2 mg/kg DM CocE reducing the lethal effects of 1000.0 mg/kg; IP cocaine by 40%. In addition, DM CocE was shown to provide a long-lasting protection against the toxic effect of cocaine, with a large dose of DM CocE capable of producing a 50% reduction in the lethal effects of 180.0 mg/kg; IP cocaine for up to 4 hours. Perhaps more importantly, these studies also demonstrated the capacity of DM CocE to dose-dependently rescue rats from the cardiovascular and lethal effects of cocaine when administered up to 1 minute after the onset of 180.0 mg/kg; IP cocaine-induced convulsion, with a 3 mg/kg dose of DM CocE saving 93% of male rats and 100% of female rats tested. In addition, a low dose of DM CocE was also shown to protect against and reverse the cardiovascular effects associated

with a relatively low dose of 5.6 mg/kg; IV cocaine. When taken together with the results of previous studies in which DM CocE was shown to dose-dependently and rapidly ameliorate the cardiovascular effects of cocaine in rhesus monkeys (Collins et al., 2011), the results of the studies reported herein provide strong evidence that DM CocE is capable of rescuing animals from the toxic effects associated with a wide range of cocaine doses and routes of administration. In conclusion, the results of these studies provide strong support the further development of highly efficient cocaine hydrolyzing enzymes, such as DM CocE, for the treatment of acute cocaine toxicity in humans.

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Figure 1.

Protective effects of DM CocE against cocaine-induced convulsion and lethality. One of three doses of DM CocE (0.32, 1.0, or 3.2 mg/kg), or PBS was administered IV 1 minute prior to an IP injection of cocaine. A) Dose-response curves for cocaine-induced convulsion in the absence or presence of DM CocE. B) Dose-response curves for cocaine-induced lethality in the absence or presence of DM CocE. Data represent the percent of rats [$n=10$ (5 male and 5 female) per dosing condition] that displayed convulsion or lethality within the 60-minute period immediately following cocaine administration.

Figure 2.

Protective effects of DM CocE against cocaine-induced convulsion and lethality. One of four doses of DM CocE (0.32, 1.0, 3.2, or 10.0 mg/kg), or PBS was administered IV at one of five times (1, 60, 120, 240, or 360 minutes) prior to an IP injection of 180.0 mg/kg IP cocaine. Dose-response analysis of the protective effects of DM CocE against cocaine-induced A) convulsion and B) lethality when administered at various time points prior to an ED_{100conv} and LD₁₀₀ dose of 180.0 mg/kg; IP cocaine. Data represent the percent of rats [n=10 (5 male and 5 female) per dosing condition] that displayed convulsion or lethality within the 60-minute period immediately after cocaine administration. *, p<0.05; ***, p<0.001. Significant differences in the percent occurrence of convulsion or lethality between

the DM CocE and PBS treated groups at each time point were determined using one-tailed fisher's exact probability tests.

Figure 3.

Protective effects of DM CocE against cocaine-induced changes in mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm). Baseline measures of MAP and HR were collected for 30 minutes prior to an IV pretreatment with PBS or 0.32 mg/kg DM CocE. Cocaine (5.6 mg/kg; IV) was administered 1 minute later, with cardiovascular measures collected for at least 20 minutes thereafter. Data are expressed as the mean $\pm$ SEM (n=6/group) change in MAP or HR from baseline (1-minute bin immediately prior to PBS or DM CocE). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant differences from PBS condition were determined using two-way ANOVA with post-hoc Bonferroni tests.

1-min Post-Convulsion Treatment

Cocaine Dose (180.0 mg/kg; IP)

Figure 4.

Capacity of DM CocE to rescue rats from the lethal effects of 180.0 mg/kg IP cocaine. Effects of DM CocE (0.32, 1.0, or 3.2 mg/kg) or PBS on the percent of rats experiencing cocaine-induced lethality when administered 1 minute after the onset of 180.0 mg/kg IP cocaine-induced convulsion. Data represent the percent of rats [$n=10$ (5 male and 5 female) per dosing condition] in which lethality was observed within the 60-min period immediately following the administration of cocaine. ***, $p<0.001$ as determined using one-tailed fisher's exact probability tests.

Figure 5.

Capacity of DM CocE to rescue rats from the cardiovascular effects of 180.0 mg/kg; IP cocaine. Baseline measures of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) were collected for 30 minutes prior to the IP administration of 180.0 mg/kg cocaine. PBS or 3 mg/kg DM CocE were administered IV, immediately after the onset of cocaine-induced convulsion, with cardiovascular measures collected for at least 20 minutes thereafter. Data are expressed as the mean $\pm$ SEM ($n=10$ /group) change in MAP or HR from baseline (1-minute bin immediately prior to cocaine administration). Numbers in parentheses represent the number of rats remaining in the group at each timepoint following the first incidence of lethality. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; #, $p<0.05$; ##, $p<0.01$;

###, $p < 0.001$. Significant differences from baseline for the DM CocE (*) and PBS (#) groups were determined using one-way ANOVA with post-hoc Dunnett's tests.

Figure 6.

Capacity of DM CocE to ameliorate the cardiovascular effects of 5.6 mg/kg; IV cocaine. Baseline measures of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) were collected for 30 minutes prior to the IV administration of 5.6 mg/kg cocaine. PBS or 0.32 mg/kg DM CocE were administered IV, 10 minutes after cocaine with cardiovascular measures collected for at least 20 minutes thereafter. Data are expressed as the mean $\pm$ SEM ($n=6$ /group) change in MAP or HR from baseline (1-minute bin immediately prior to cocaine administration). *, $p<0.05$; **, $p<0.01$. Significant differences from PBS condition were determined using two-way ANOVA with post-hoc Bonferroni tests.

Effects of DM CocE on the percent incidence of convulsion and lethality in male and female rats induced by different doses of cocaine

Table 1

Cocaine mg/kg; IP	DM CocE Dose (mg/kg; IV)									
	0.0 (PBS)		0.32		1.0		3.2			
	Male C ^a /L ^b	Female C ^a /L ^b	Male C ^a /L ^b	Female C ^a /L ^b	Male C ^a /L ^b	Female C ^a /L ^b	Male C ^a /L ^b	Female C ^a /L ^b	Male C ^a /L ^b	Female C ^a /L ^b
0.0	0/0	0/0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32.0	0/0	0/0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
56.0	60/40	100/40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100.0	80/80	100/100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
180.0	100/100	100/100	40/40	0/0	0/0	0/0	0/0	0/0	0/0	0/0
320.0	n.d.	n.d.	100/80	80/60	0/0	20/0	20/0	20/0	20/0	20/20
560.0	n.d.	n.d.	100/100	100/100	80/80	100/100	100/100	100/100	100/100	100/80
1000.0	n.d.	n.d.	100/100	100/100	80/80	80/80	40/40	40/40	40/40	100/80

^aPercent incidence of convulsion
^bPercent incidence of lethality
n.d. = not determined

Effects of DM CocE on the percent incidence of convulsion and lethality in male and female rats when administered at various times before 180.0 mg/kg; IP cocaine

Table 2

PT Time (min)	DM CocE Dose (mg/kg; IV)									
	0.0 (PBS)		0.32		1.0		3.2		10.0	
	Male C ^a / L ^b	Female C ^a / L ^b	Male C ^a / L ^b	Female C ^a / L ^b	Male C ^a / L ^b	Female C ^a / L ^b	Male C ^a / L ^b	Female C ^a / L ^b	Male C ^a / L ^b	Female C ^a / L ^b
1	100 / 100	100 / 100	60 / 60	100 / 80	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
60	100 / 100	100 / 100	100 / 100	100 / 100	80 / 40	100 / 100	20 / 0	0 / 0	0 / 0	0 / 0
120	100 / 100	100 / 100	100 / 100	100 / 100	100 / 100	80 / 80	60 / 60	100 / 80	0 / 0	0 / 0
240	100 / 100	100 / 100	100 / 100	100 / 100	100 / 80	100 / 100	100 / 100	100 / 100	20 / 20	60 / 60
360	100 / 100	100 / 100	100 / 100	100 / 100	100 / 100	100 / 80	100 / 80	100 / 100	100 / 100	100 / 100

^aPercent incidence of convulsion

^bPercent incidence of lethality

Table 3

Capacity of DM CocE to recover male and female rats from 180.0 mg/kg; IP cocaine-induced acute toxicity

	DM CocE Dose (mg/kg; IV)			
	0.0 (PBS)	0.32	1.0	3.2
Male (C^a / L^b)	100 / 100	100 / 100	100 / 40	100 / 20
Female (C^a / L^b)	100 / 100	100 / 60	100 / 0	100 / 0

^aPercent incidence of convulsion^bPercent incidence of lethality