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A Bacterial Cocaine Esterase Protects Against Cocaine-Induced Epileptogenic Activity and Lethality

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Abstract

Study objective—Cocaine toxicity results in cardiovascular complications, seizures, and death and accounts for approximately 20% of drug-related emergency department visits every year. Presently, there are no treatments to eliminate the toxic effects of cocaine. The present study hypothesizes that a bacterial cocaine esterase with high catalytic efficiency would provide rapid and robust protection from cocaine-induced convulsions, epileptogenic activity, and lethality.

Methods—Cocaine-induced paroxysmal activity and convulsions were evaluated in rats surgically implanted with radiotelemetry devices (N=6 per treatment group). Cocaine esterase was administered 1 minute after a lethal dose of cocaine or after cocaine-induced convulsions to determine the ability of the enzyme to prevent or reverse, respectively, the effects of cocaine.

Results—The cocaine esterase prevented all cocaine-induced electroencephalographic changes and lethality. This effect was specific for cocaine because the esterase did not prevent convulsions and death induced by a cocaine analog, (–)-2β-carbomethoxy-3β-phenyltropane. The esterase prevented lethality even after cocaine-induced convulsions occurred. In contrast, the short-acting benzodiazepine, midazolam, prevented cocaine-induced convulsions but not the lethal effects of cocaine.

Conclusion—The data showed that cocaine esterase successfully degraded circulating cocaine to prevent lethality and that cocaine-induced convulsions alone are not responsible for the lethal effects of cocaine in this model. Therefore, further investigation into the use of cocaine esterase for treating cocaine overdose and its toxic effects is warranted.

INTRODUCTION

Background

At high doses, cocaine produces a number of toxic effects, leading to more than 125,000 emergency visits, or approximately 20% of all drug-related emergency department (ED) visits annually.¹ Cocaine toxicity results in cardiovascular complications, seizures, and death. It has also been suggested that respiratory depression plays a causative role in cocaine-induced death.^{2–5}

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Author contributions: EMJ and JHW conceived the study and designed the experiments. EMJ, MGB, and ZDC performed the data collection and analysis. DN and RKS produced and purified the cocaine esterase used in this study. EMJ drafted the article, and all authors contributed to the revisions. EMJ and JHW take responsibility for the paper as a whole.

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Currently, there are no drugs available to reverse all of the effects of cocaine, and ED treatments for cocaine toxicity aim to minimize symptoms.⁶ Cardiovascular complications include chest pain, hypertension, arrhythmias, coronary vasoconstriction, and myocardial ischemia.⁷ Nitroglycerin, calcium channel blockers, the α -blocker phentolamine, and β -blockers (under some circumstances) are recommended, and the administration of aspirin also inhibits platelet aggregation during myocardial ischemia. Benzodiazepines are administered to decrease seizures and control agitation, and sodium bicarbonate and mechanical ventilation are used to reverse acidosis. Managing cocaine toxicity is complex and requires close monitoring because of the prolonged actions of high doses of cocaine.

Importance

One approach to minimize cocaine's toxic effects is to increase the rate of degradation by the administration of either an anticocaine catalytic or noncatalytic antibody or a cocaine-metabolizing enzyme. However, both the anticocaine catalytic antibody (monoclonal antibody 15A10),^{8–10} butyrylcholinesterase, and several butyrylcholinesterase mutants have low catalytic efficiency, offering insufficient protective effects against cocaine toxicity.^{11–15} A bacterial cocaine esterase (CocE) found in *Rhodococcus* sp. MB 1 living in soil surrounding the coca plant has high efficiency for degrading cocaine^{10,16} and, similar to endogenous butyrylcholinesterase, it hydrolyzes the benzoyl ester of cocaine to produce metabolites, ecgonine methyl ester and benzoic acid.¹⁰ The catalytic efficiency of CocE is sufficient to protect against the toxic and lethal effects of cocaine, as demonstrated in rats and mice^{15,17}; however, little is known about the effects of the esterase on other cocaine-related pathophysiologic changes. Therefore, the present study investigated the effects of CocE on cocaine-induced epileptiform activity and observable convulsions in relation to lethality.

Goals of This Investigation

The present study hypothesized that the bacterial CocE would protect against the toxic effects of cocaine, specifically epileptiform activity, overt convulsions, and death, in rats. High doses of cocaine produce overt behavioral convulsions and simultaneous epileptogenic activity in animals and humans.^{4,18–23} In the current study, the effects of CocE on cocaine-induced convulsions, electroencephalographic (EEG) activity, and lethality were evaluated by telemetry measurements in freely moving rats. EEG activity was evaluated in addition to overt behavioral convulsions to assess any potential nonconvulsive seizure activity that could be occurring with or instead of convulsions. The selectivity of CocE for cocaine was evaluated in rats that received a cocaine analog ((-)-2 β -carbomethoxy-3 β -phenyltropane [WIN-35,065-2])²⁴ lacking CocE's site of action, the benzoyl ester. In addition, the effects of CocE were compared with those produced by the short-acting benzodiazepine midazolam on cocaine-induced seizurogenic activity.

MATERIALS AND METHODS

Study Design

Experiment 1—Rats were implanted with the EEG radio transmitters and allowed to recover for 5 to 7 days. Then rats were implanted with intravenous catheters and allowed to recover for 4 to 5 days. After this second recovery period, the effects of CocE were evaluated on cocaine- or WIN-35,065-2-induced convulsions, seizures, and lethality. Rats were administered a lethal dose of cocaine (180 mg/kg) or WIN-35,065-2 (560 mg/kg) intraperitoneally as determined from previous studies.¹⁵ CocE or phosphate-buffered saline solution was administered intravenously 1 minute after cocaine or WIN-35,065-2. There were 4 different treatment groups (cocaine+phosphate-buffered saline solution, cocaine

+CocE, WIN-35,065-2+phosphate-buffered saline solution, WIN-35,065-2+CocE), and 6 rats were used per treatment group, for a total of 24 rats in this experiment.

Experiment 2—Another group of rats were implanted with the EEG radio transmitters and allowed to recover for 5 to 7 days. Then these same rats were implanted with intravenous catheters and allowed to recover for 4 to 5 days. Subsequently, the ability of CocE to rescue rats after a cocaine-induced convulsion was evaluated. For this experiment, rats were administered cocaine (180 mg/kg) intraperitoneally, and CocE or phosphate-buffered saline solution was administered intravenously immediately after the end of a cocaine-induced convulsion or 1 minute after the end of the cocaine-induced convulsion. There were 4 different treatment groups (cocaine+phosphate-buffered saline solution immediately after conv, cocaine+CocE immediately after convulsion, cocaine+phosphate-buffered saline solution 1 minute after conv, cocaine+CocE 1 minute after conv), and 6 rats were used per treatment group, for a total of 24 rats used in this experiment.

Experiment 3—In another set of experiments, the effects of multiple doses of midazolam on cocaine-induced convulsions and lethality were evaluated in naïve rats. This experiment identified the dose of midazolam that adequately prevented convulsions or death that could be used in the next study (experiment 4). In each rat, a single dose of midazolam (0, 0.32, 1, or 3.2 mg/kg) was administered subcutaneously as a 15-minute pretreatment to 180 mg/kg cocaine (intraperitoneally). Six rats per group were tested with 0 or 0.32 mg/kg midazolam, 8 rats received 1 mg/kg, and 5 rats were treated with 3.2 mg/kg midazolam.

Experiment 4—Rats were implanted with the EEG radio transmitters and allowed to recover for 5 to 7 days. After the recovery period, the effects of midazolam (1.0 mg/kg) were evaluated on cocaine-induced seizure activity, convulsions, and lethality. Midazolam (1.0 mg/kg, subcutaneously) was administered 15 minutes before cocaine (180 mg/kg, intraperitoneally). Six rats were tested with this treatment regimen.

Male Sprague-Dawley rats (250–350g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed in groups of 3 animals per cage on arrival. Food and water were freely available for all rats, and the housing and experimental rooms were maintained on a 12-hour light/dark cycle, with lights on at 7 AM at an average temperature of 21°C. Rats were habituated to the laboratory environment for approximately 7 days before use. After surgical procedures, rats were housed singly for the duration of the experiment. All rats received only 1 treatment condition. If a rat did not die during an experiment, it was euthanized 24 hours after the end of an experiment. The experimental protocols were approved by the University of Michigan University Committee on the Use and Care of Animals and conformed to the guidelines established by the *NIH Guide for the Use of Laboratory Animals*.²⁵

Methods of Measurement

Electroencephalograms were measured with a telemetry system through 3-channel radio transmitters (model F20-EET; Data Sciences International, St. Paul, MN). Transmitters were implanted surgically under ketamine (90 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally) anesthesia. The transmitter was 22 mm long, 10 mm wide, and 10 mm deep and weighed approximately 4.3 g. Before implantation, the transmitter was cleaned in ethanol and soaked in sterile saline solution. An incision in the skin and musculature of the peritoneal cavity was made, and the transmitter was placed inside the peritoneal cavity. The transmitter was attached to the muscle wall of the peritoneum with nonabsorbable nylon suture to prevent the transmitter from shifting after implantation, and the skin over the muscle was closed. The biopotential leads (4) were passed through the muscle wall of the

peritoneum with a 16-gauge needle and threaded subcutaneously, emerging at an incision made in the skin at the base of the head. The rat's head was placed in a stereotaxic instrument for screw and biopotential lead attachment. After exposure of the skull, the bone was cleaned and dried. Four holes were drilled with a microdrill with 0.7-mm steel burr (Fine Science Tools, Inc., Foster City, CA) for bilateral placement of epidural recording electrodes, which consisted of biopotential leads projecting from the transmitter wrapped around stainless steel slotted, fillister screws (0.8-mm diameter, 0.12 in long; Small Parts, Inc., Miami Lakes, FL). The biopotential leads were prepared by removing approximately 0.5 cm of silicone rubber tubing from the end of each wire, and the wires were stretched to allow easier and more secure wrapping around the skull screws. These recording electrodes were implanted over the left and right parietal cortex (each side approximately 1 mm posterior to bregma, 1.5 mm lateral to the midline and 1 mm anterior to lambda, 1.5 mm lateral to the midline). All skull screws and wires were anchored to the skull with dental acrylic cement. After biopotential lead attachment, the skin incisions were closed with nylon suture. After surgery, rats were housed singly and monitored daily for signs of recovery.

Catheters were constructed from approximately 15 cm of Micro-Renathane tubing (MRE-040; Braintree Scientific, Inc., Braintree, MA). Briefly, rats were anesthetized as described above and the right jugular vein was isolated through a ventral incision in the neck. Approximately 2.5 to 3 cm of the catheter (depending on the size of the rat) was inserted into the right jugular vein. The tubing was sutured to the vein and to the surrounding tissues at 3 to 4 points to secure the catheter placement. The remaining tubing was threaded subcutaneously, passed outside the body through a dorsal incision point, and secured in place by suturing to musculature directly below the incision. Two to three centimeters of tubing remained exposed outside the rat's body and was plugged with a stainless steel pin. Every day after the surgery, the catheters were flushed with approximately 0.5 mL of heparinized saline (50 U/mL). After this second surgery, rats remained single-housed.

For intraperitoneal and subcutaneous injections, rats were lightly restrained for approximately 5 to 10 seconds during the injection. For intravenous infusions, rats were allowed to freely move around their home cage while the experimenter removed the catheter pin, attached a blunted needle with syringe, and infused the solution during 30 seconds. This was followed by a heparinized saline solution flush (0.3–0.5 mL) and replacement of the catheter pin.

For all EEG implant experiments, bilateral, cortical EEGs were collected. Baseline EEG activity was recorded continuously for at least 1 hour before handling and throughout the rest of the experiment. Recordings were collected from one rat at a time to allow continual observations and synchronizing of behavioral changes with EEG disturbances. After all drug injections, rats were observed continuously for any behavioral or physiologic changes, including changes in locomotor activity, convulsions, pre-ictal activity, myoclonic twitches, and death. Death was defined by a visual lack of diaphragm movement, with a lack of heartbeat as measured by touch (measured with forefinger and middle finger only). Descriptions of behavioral changes were documented against the EEG software clock to link behavioral changes with EEG alterations. EEG traces were not analyzed at this time; only changes in normal EEG patterns and behavior were noted for later analysis. Testing treatments were randomized across the experimental days, and all experiments were conducted between 9 AM and 2 PM.

Rats not implanted with radio transmitters were used for analyzing the dose effect curve for midazolam pretreatment on cocaine-induced convulsions and lethality. Rats were placed individually into clean observation cages. Each rat was administered saline solution or 0.32,

1.0, or 3.2 mg/kg midazolam (subcutaneously) as a 15-minute pretreatment to 180 mg/kg cocaine (intraperitoneally). Rats were observed continuously for behavioral changes, including sedation, muscular lethargy, myoclonic twitches, convulsions, and death.

Data Collection and Processing

Signals detected by the biopotential leads were transmitted to a receiver (RPC-1; Data Sciences International) beneath each rat's home cage. The receiver sent the signal through a cable connector to the Dataquest ART Exchange Matrix (Data Sciences International) that converted the analog signal into digital output that was recorded onto a computer. The signal was filtered for 60-Hz power-line signal, and the low-pass filter was set to 0.5 Hz and the high-pass filter was fixed at 70 Hz. Electroencephalograms were analyzed visually at a 30 mm per second recording speed with Somnologica Software and DSI import modules (Medcare Flaga, Reykjavik, Iceland). All EEG traces were recorded bilaterally; however, an EEG trace from one hemisphere only is shown in each figure for simplicity. The baseline amplitude for all traces shown is approximately 100 μ V.

Outcome Measures and Primary Data Analysis

All subjects were observed continually from the time of injection until death or for 2 hours after a cocaine injection. The main outcomes measured for this study were seizure activity, overt behavioral convulsions, and lethality. Behavioral convulsions were determined by visual observation. Lethal effects were identified by the simultaneous lack of breathing movements and heartbeat as determined by observation and feeling for a heartbeat over the ribcage. The presence of convulsions and lethality with or without CocE were analyzed by Fisher's exact test or χ^2 tests (GraphPad Prism Software, La Jolla, CA).

Seizure activity was measured by visual analysis of the electroencephalograms by 2 raters independently, one blind and one not blind to the experimental treatments. Interrater reliability was greater than 90%. Seizure activity was defined as a discharge sequence that increased in amplitude and changed in frequency as compared with baseline, consisting of rhythmic spikes, sharp waves, and spike-and-wave complexes. These discharge sequences also had to occur in the frequency range of 0.5 to 3.5 Hz (delta frequency) by spectral analysis provided by the software program (Somnologica Software; Medcare Flaga).

The secondary outcomes measured were other behavioral changes such as increases in locomotor activity, changes in muscle tone and breathing rate, and characteristics of seizure and seizure-like activity, such as tremors, clonus, tonus, rearing, falling down, and myoclonic twitches. In addition to the primary ictal activity analyzed in the electroencephalograms, other possible secondary outcomes (postictal suppression and pre-seizure amplitude changes) were evaluated as compared with baseline EEG activity.

(–)-Cocaine was obtained from the National Institute on Drug Abuse (Bethesda, MD). WIN-35,065-2 was provided by Dr. F. Ivy Carroll (National Institute on Drug Abuse, Research Triangle Institute, NC). Cocaine and WIN-35,065-2 were dissolved in sterile water. Midazolam (Bedford Laboratories, Bedford, OH) was provided by the National Institute on Drug Abuse and diluted in saline solution. All drugs were administered in a volume of 1 to 1.5 mL/kg.

RESULTS

Figure 1 illustrates the chemical structures of cocaine and a cocaine analog, WIN-35,065-2. Descriptive characteristics of cocaine- and WIN-35,065-2-induced convulsions and lethality are reported in Table 1. The doses of cocaine and WIN-35,065-2 (180 mg/kg and 560 mg/kg, respectively) were chosen for this study because they were the smallest doses (in quarter

logarithm increments) that produce lethality in all rats tested. Although these compounds are close chemical analogs, there were some qualitative and quantitative pharmacologic differences between them. For example, WIN 35,065-2 produced more convulsions per rat that were slower in onset and longer in duration compared with cocaine. Similarly, the lethal effects of cocaine were faster and more consistent compared with those of WIN 35,065-2.

Figure 2 shows representative electroencephalograms after an injection of 180 mg/kg cocaine (intraperitoneally). Cocaine administration did not induce changes in EEG activity before the convulsion, such that EEG activity 1 minute before the convulsion (Figure 2C) was similar to normal activity (Figure 2B). Although there were no EEG changes before a convulsion, all rats demonstrated behavioral activation, including increased locomotion followed by loss of balance and muscle tone, increased respiration rate, and increased reactions to noise or handling.

The cocaine-induced convulsion was correlated with bilateral ictal patterns of spike-and-wave complexes measured over the parietal cortex (Figure 2D). The convulsion consisted of forelimb clonus in 6 of 6 rats and progressed to generalized clonic convulsions in 3 of 6 rats. After the convulsion, all rats remained unconscious and EEG activity was suppressed dramatically and did not recover. Five of 6 rats demonstrated postconvulsive myoclonic twitches corresponding with an isolated sharp wave, polyspike, or spike-and-wave complex (Figure 2E). Within a few minutes after a convulsion, all EEG activity ceased and a heartbeat was no longer detectable in all rats tested (Figure 2F).

Figure 3 shows representative traces demonstrating the effects of CocE administered 1 minute after cocaine injection on cocaine-induced convulsions. Similar to data shown in Figure 2, cocaine (180 mg/kg) followed by an intravenous injection of phosphate-buffered saline solution produced a convulsion and corresponding ictal activity followed by postconvulsive myoclonic twitches and EEG suppression with discrete discharges (Figure 3A). However, CocE prevented all cocaine-induced ictal and postictal EEG changes, as well as all overt convulsions and lethality (Table 2; $P=.002$ for both measurements compared with phosphate-buffered saline solution), such that EEG activity immediately and 1 hour after cocaine administration was identical to baseline recordings (Figure 3C and D). In all rats receiving 1 mg CocE intravenously, 180 mg/kg cocaine failed to produce seizures and ictal discharges (Figure 3B). Although cocaine did not produce EEG changes, convulsions, or lethality in esterase-treated subjects, the rats demonstrated cocaine-induced behavioral changes, such as sensitivity to tactile and auditory stimuli, increased respiration levels, and loss of muscle tone. Approximately 30 to 50 minutes after esterase administration, rats demonstrated increased locomotion and stereotypy.

As described in Table 1, 560 mg/kg WIN-35,065-2 produced convulsions in all rats tested that occurred more frequently and lasted longer than cocaine-induced convulsions, but they also appeared more intense and forceful. Before convulsion, full-body tremors and head bobbing were observed. Convulsions consisted of forelimb clonus, generalized clonic convulsions, tonic convulsions, rearing, falling down, and stiff tail. In between convulsive episodes, myoclonic twitches, tremors, and altered respiratory rates were observed in all rats.

WIN-35,065-2 also produced a different profile of EEG activity compared with cocaine. Figure 4A and D and Figure 5A show representative EEG traces from individual rats that received 560 mg/kg WIN35063-2 followed by an intravenous injection of phosphate-buffered saline solution. The observed convulsions correlated with episodes of ictal activity with increased amplitude and decreased frequency and consisted of sharp waves, spikes, polyspikes, and some spike-and-wave complexes (Figure 4B). As the intensity of the overt

behavioral convulsion decreased, the ictal activity varied in amplitude and frequency, with no identifiable pattern or rhythmicity (Figure 4C). Immediately after each convulsion, EEG activity was either similar to baseline or slightly slowed, with occasional spikes or sharp waves corresponding with myoclonic twitches.

The effects of CocE on WIN-35,065-2-induced epileptiform activity, convulsions, and death are shown in Figure 5 and Table 2. Esterase administration did not alter the convulsive characteristics of WIN-35,065-2, producing similar mean number of convulsive episodes (4 with range 1–14) and mean time to death (622 seconds, with range 276–1324) as produced by WIN-35,065-2+ buffered saline solution (Table 1). In addition, rats receiving CocE 1 minute after WIN-35,065-2 demonstrated the same convulsive profile and epileptiform activity compared with rats that received phosphate-buffered saline solution instead of the esterase (Figures 4A and D and 5A). In addition, CocE did not prevent WIN-35,065-2-induced behavioral convulsions or lethality in any rats tested (Table 2).

Thus far, these data have demonstrated that CocE administered after cocaine injection prevented cocaine-induced convulsions and lethality. Figure 6 also demonstrates that CocE can prevent cocaine-induced lethality even when administered after the cocaine-induced convulsion. As expected, phosphate-buffered saline solution administered immediately after cocaine-induced convulsions did not alter the number of rats that died, the time to cocaine-induced death, or the EEG activity before death (Figure 6A and Table 2). When the esterase was administered after the overt behavioral convulsion ended, all rats survived (N=6) (Table 2; $P=.002$ compared with phosphate-buffered saline solution). Figure 6B demonstrates a representative EEG trace from a rat that recovered after convulsion induced by 180 mg/kg cocaine. After the epileptiform activity, postictal EEG changes were observed, such as disrupted EEG activity with varying amplitude and low frequency. Within 3 minutes after the end of the cocaine-induced convulsion, the EEG activity returned to baseline levels, but the rats demonstrated stereotypy and increased locomotor activity levels.

When the esterase was administered 1 minute after the end of the cocaine-induced convulsion, 67% of rats (4 of 6) tested were rescued (Table 2; $P=.061$ compared with phosphate-buffered saline solution). Figure 6C shows a representative trace from a rat that received CocE 1 minute after the end of the cocaine-induced convulsion. In the rescued rats, EEG activity returned to baseline levels within approximately 1 minute of receiving the esterase, and stereotypy and locomotor activity were increased after recovery from the postconvulsive EEG changes. In the rats that were not rescued by CocE, respiratory rates decreased dramatically immediately after the convulsion, and esterase administration did not reverse these effects.

As a comparison to CocE, the protective effects of the benzodiazepine midazolam were evaluated in rats treated with a lethal dose of cocaine. Midazolam administered as a 15-minute pretreatment to 180 mg/kg cocaine significantly decreased cocaine-induced convulsions ($P<.001$) and lethality ($P=.035$) (Figure 7A). High doses of midazolam were required to attenuate convulsions in all rats tested, but they did not completely prevent lethality, such that approximately 60% of rats lived with 1.0 mg/kg (3 of 8 died) and 3.2 mg/kg (2 of 5 died) midazolam.

Although midazolam blocked observable convulsions produced by cocaine, nonconvulsive seizures could occur in the presence of midazolam, contributing to the lethal effects. Therefore, cocaine-induced EEG changes were measured after midazolam pretreatment. Midazolam alone suppressed locomotor activity and induced loss of muscle tone that correlated with periodic slowing of the EEG trace, as demonstrated by a decreased frequency or increased amplitude (Figure 7D and E compared with Figure 7C before

midazolam). Cocaine administration slightly increased activity levels and respiratory rates, although the rats had low muscle tone and were unstable. EEG changes began occurring approximately 5 minutes after cocaine administration (Figure 7B). Although there were no observable convulsions, EEG changes occurred with occasional, brief episodes (1 second or less) of increased amplitude and decreased frequency that became more frequent over time. In the last 1 to 2 minutes before death (Figure 7F), the EEG activity was suppressed, with no identifiable pattern. In one rat, a few myoclonic twitches correlated with spike-and-wave complexes that occurred after respiration stopped 23 seconds before death (no heartbeat). However, no specific ictal activity was observed in any other rat tested before death. Death occurred at a mean time of 622.4 seconds (range 432 to 931 seconds) after cocaine administration, 3.3 times longer than without midazolam pretreatment (compared with times in Figure 1).

LIMITATIONS

Some of the major limitations of this study involve the applicability of these experiments to the clinical overdose situation. First, the present study used a massive, acute dose of cocaine to induce epileptogenic activity, convulsions, and lethality. With the exception of cocaine body-packing,²⁶ most overdose circumstances do not occur after a single exposure to a massive cocaine dose but occur in chronic cocaine users. Long-term cocaine use produces cardiovascular and cerebrovascular damage over time, and instances of overdose may be due to the combination of circulating cocaine levels with accumulated cardiovascular damage. The present study may not be relevant to this clinical scenario of long-term cocaine use and subsequent overdose; therefore, CocE may not be as effective in treating cocaine overdose as proposed in the present study. For example, CocE may eliminate high levels of circulating cocaine and may prevent lethality even after cocaine-induced convulsions occur but may not reverse the cocaine-initiated cardiotoxic events (ie, ischemia). Future studies will need to evaluate the effects of CocE in clinically relevant cocaine-overdose models.

This leads to the second limitation that the present study did not evaluate simultaneously the effects of CocE on cocaine-induced cardiovascular changes and cardiotoxic events. The massive doses of cocaine used in the present study likely produced dysrhythmias and ischemia, precipitating seizures and lethality, and the effects of CocE on these cardiac changes are not known. Even in instances in which the esterase prevented lethality or seizures, cocaine exposure may have produced undetected cardiovascular damage. A future study will report on the effects of CocE on cocaine-induced cardiovascular changes leading to lethality.

Despite these limitations, this study demonstrated that CocE has highly efficient and fast-acting catalytic activity in vivo to eliminate massive, circulating doses of cocaine that normally produce seizures and lethal effects. This study and others^{15,17} serve as the basis for future studies on the use of CocE for cocaine overdose and demonstrate that further investigation is warranted.

DISCUSSION

In the present study, the bacterial CocE was used to increase degradation of lethal cocaine doses. CocE prevented cocaine-induced convulsions and lethality similar to that shown previously in rats and mice^{15,17}; in addition, CocE attenuated all epileptogenic activity associated with administration of a lethal cocaine dose. The esterase administered 1 minute after cocaine abolished cocaine-induced seizurogenic activity, overt behavioral convulsions, and lethality. In the absence of observable convulsions, cocaine failed to produce any changes in cortical EEG activity; however, some undetectable, focal EEG changes might

have occurred. CocE significantly decreased cocaine levels to eliminate convulsions, paroxysmal activity, and death; however, circulating cocaine levels after CocE most likely remained high, considering stereotypy and locomotor stimulation were observed for hours. Although CocE has a superior catalytic efficiency in vitro,¹⁶ the extent of cocaine metabolism in vivo by CocE is unknown. At 37°C in vitro, the esterase had a short half-life (≈ 13 min) and decreased cocaine levels by 150-fold within 1 minute in cocaine-spiked, human plasma samples¹⁵; however, this activity may be insufficient to eliminate completely large, toxic doses of cocaine in vivo. Alternatively, the remaining behavioral effects (eg, stereotypy and locomotor stimulation) could be related to cocaine metabolites produced by CocE. Future studies will evaluate the circulating levels of cocaine and cocaine metabolites after CocE administration in vivo to determine whether greater doses, repeated administration, or a longer-acting mutant of CocE would provide improved cocaine elimination. These studies would indicate the level of elimination required to reverse all symptoms of cocaine toxicity and actions of cocaine to enhance therapeutic actions.

Esterase treatment prevented all cocaine-induced epileptogenic activity and death. In preclinical studies, no other treatment has been reported to have this impressive an effect against an acute, large cocaine dose. Currently, the main pharmacologic interventions for cocaine toxicity include the administration of anticonvulsants (benzodiazepines), calcium channel blockers, adrenergic antagonists, and sodium bicarbonate. The benzodiazepine diazepam blocked cocaine-induced convulsions but only minimized paroxysmal activity and partially prevented the lethal effects of cocaine.^{18,27} Similarly, in the present study, the short acting-benzodiazepine, midazolam, eliminated overt convulsions and dramatically lessened seizurogenic activity, without completely preventing lethality. The glutamate *N*-methyl-D-aspartate receptor antagonist MK801 (dizocilpine) and valproic acid also minimized cocaine-induced convulsions but did not alter lethality. Interestingly, in these studies, using mechanical ventilation in MK801- or valproic acid-treated rats enhanced survival but also induced the revival of paroxysmal EEG activity, demonstrating that reversal of hypoxemia and acidosis revived cerebral activity and normal, cocaine-induced EEG changes.⁴

Cardiovascular interventions aim to minimize cardiotoxic events, such as myocardial ischemia, coronary vasoconstriction, ventricular fibrillation, reductions in coronary blood flow, and microvascular spasms.^{28–33} Administration of either a calcium channel blocker or the β -adrenergic antagonist propranolol with diazepam blocked convulsions and reduced epileptogenic activity but did not significantly attenuate death.¹⁸ Similarly, the α_1 -adrenergic antagonist prazosin slightly attenuated cocaine-induced lethality, whereas β -adrenergic antagonists enhanced lethality.³⁴ Calcium channel antagonists failed to provide significant protection from cocaine-induced lethality.^{18,34} Overall, these data suggest that cocaine-induced seizures alone are not responsible for the lethal effects and further demonstrate that cardiotoxic events and subsequent respiratory depression contribute to the lethal effects of cocaine. As previously stated, none of the above-described treatments were able to prevent cocaine-induced convulsions, epileptogenic changes, and lethality to a similar extent as demonstrated with CocE in the present study.

CocE administered immediately after a cocaine-induced convulsion also prevented death in all rats tested. Under these circumstances, normal EEG activity returned within 3 minutes of the end of the convulsion, and the rats were behaviorally stimulated in terms of stereotypy and locomotor activity. Similarly, administration of CocE after a cocaine-induced convulsion rescued mice from death and dose-dependently shortened the recovery time after the end of the convulsion.¹⁷ Interestingly, CocE administered 1 minute after the end of the convulsion also prevented death in 67% of the rats tested. In the 33% of rats not rescued, respiration decreased dramatically after the end of the convulsion, suggesting that the toxic effects become irreversible or unrecoverable in some animals. These data demonstrate that

cocaine toxicity is a complicated process involving respiratory depression, cardiovascular toxicity, and seizures, and eliminating high cocaine levels may not be sufficient to reverse extensive damage that occurs during cocaine exposure.^{3,4}

Cocaine and the cocaine analog WIN-35,065-2 produced convulsions, epileptiform activity, and death; however, the behavior manifestation and EEG activity varied significantly between these 2 compounds. It was previously demonstrated that cocaine and WIN-35,065-2 produced similar effects on operant behaviors, although the compounds differed in potency.^{35,36} In the present study, the 100% lethal doses of cocaine and WIN-35,065-2 differed by approximately 3-fold. The lethal dose of cocaine (180 mg/kg) produced 1 convulsion before death, whereas this lethal dose of WIN-35,065-2 (560 mg/kg) stimulated multiple convulsive episodes before death. A quarter-log lower dose of WIN-35,065-2 produced convulsions in rats with mainly clonic features but was not lethal (data not shown). The manifestation of the WIN-35,065-2-induced convulsion appeared more severe, involving tonic components and rearing during generalized clonic seizures. During a cocaine convulsion, the paroxysmal activity consisted of monomorphic, rhythmic spike-and-wave complexes, but the WIN-35,065-2 ictal pattern was not as rhythmic and polymorphic, containing more spike activity and polyspike formations, consistent with tonic-clonic (or grand mal) seizures. Although these compounds are structural analogs, they clearly have some distinctive effects. Overall, these differences suggest that WIN-35,065-2 may not be the most appropriate control compound to compare with the behavioral effects of cocaine; however, it is a satisfactory control, considering the enzymatic actions of CocE.

CocE did not alter any aspect of WIN-35,065-2-induced convulsions, seizures, or lethality. In contrast, CocE was able to completely attenuate cocaine-induced seizures and lethality. These data further demonstrate that CocE has selective activity for the cocaine structure hydrolyzing the benzoyl ester only. In addition, esterase alone (without cocaine) had no effects on observable behaviors or EEG activity. These findings suggest that CocE reversed the toxic effects of cocaine without having deleterious effects of its own. The esterase does have some potential for increasing anti-CocE antibody titers after repeated administration, which ultimately decreases the effects of CocE and could lead to immune responses.¹⁷ These concerns would need to be addressed before repeated administration in humans.

In conclusion, CocE prevented cocaine-induced convulsions and all related seizurogenic activity. CocE also attenuated the lethal effects of cocaine overdose and was able to rescue some subjects after cocaine-induced convulsions with suppressed EEG activity and decreased respiration. Overall, these data demonstrated that CocE can dramatically enhance the degradation of cocaine to reverse the toxic effects of acute, large doses of cocaine, indicating that this esterase may have potential for treating cocaine overdose, but further investigation is necessary.

Editor's Capsule Summary

What is already known on this topic

There is no agent in clinical use that eliminates cocaine from the body by degradation.

What question this study addressed

Can a cocaine esterase of bacterial origin block the clinical effects of a lethal dose of cocaine in rats?

What this study adds to our knowledge

This study suggests that this agent can prevent seizures and prevent death from cocaine in rats.

How this might change clinical practice

This trial will not affect practice but suggests that this type of agent might have a clinical use if proven safe and effective in humans.

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Figure 1.
Chemical structures for *A*, cocaine and its analog *B*, WIN35065-2.

Figure 2.

Representative EEG recordings from a rat that received a lethal dose of 180 mg/kg cocaine (intraperitoneally). The baseline amplitude for all traces is approximately 100 μ V. *A*, Trace of EEG activity immediately after an injection of 180 mg/kg cocaine until death. Vertical timelines indicate 10-second intervals. *B*, Expanded trace of the 60 seconds before cocaine injection. *C*, Expanded trace of the 60 seconds before convulsion. *D*, A 20-second trace of the cocaine-induced convulsion occurring approximately 2 min after cocaine injection. *E*, EEG trace demonstrating activity 1 minute after the end of the cocaine-induced convulsion. *F*, Part of the above EEG trace demonstrating the final 3 discharges followed by death, determined by lack of heartbeat.

Figure 3.

The effects of cocaine esterase (intravenously) on EEG changes produced by 180 mg/kg cocaine. The baseline amplitude for all traces is approximately 100 μ V. *A*, A representative trace from a rat that received 180 mg/kg cocaine (intraperitoneally, at single asterisk) following by 0.1 mL phosphate-buffered saline (PBS, intravenously, at double asterisks) 1 minute after the cocaine injection. The timelines indicate 30-second intervals. *B*, A representative trace from a rat that received 180 mg/kg cocaine (intraperitoneally) followed by 1 mg cocaine esterase (intravenously) 1 minute after the cocaine injection. The timelines indicate 5-minute intervals. *C*, An expanded trace showing baseline EEG activity 1 minute before cocaine injection. *D*, An expanded trace showing EEG activity 1 hour after cocaine injection.

Figure 4.

Representative recordings from rats receiving lethal doses of WIN-35,065-2 (intraperitoneally). The baseline amplitude for all traces is approximately 100 μ V. *A*, EEG trace from a rat that received 560 mg/kg WIN-35,065-2 (intraperitoneally) following 1 minute later by 0.1 mL PBS (intravenously). Timelines indicate 2-minute intervals. The rectangular box shows the section of trace expanded in *B* and *C*. *B*, An expanded trace of the start of the convulsion (3 cm/s, approximately 10.3 seconds) produced by 560 mg/kg WIN-35,065-2 (intraperitoneally). *C*, An expanded trace of the end of the convulsion (3 cm/s, approximately 10.3 seconds) produced by 560 mg/kg WIN-35,065-2 (intraperitoneally). *D*, Another rat that received 560 mg/kg WIN-35,065-2 (intraperitoneally) followed by PBS. Timelines indicate 30-second intervals. The single asterisk indicates the WIN-35,065-2 injection (intraperitoneally) that produced an electrical artifact as the rat was removed from the receiver, interrupting the telemetry signal. The PBS injection is identified with double asterisks.

Figure 5.

The effects of cocaine esterase (intravenously) on EEG changes produced by 560 mg/kg WIN-35,065-2 (intraperitoneally). The baseline amplitude for all traces is approximately 100 μ V. *A*, Representative trace from a rat that received 0.1 mL PBS (intravenously) 1 minute after 560 mg/kg WIN-35,065-2 (intraperitoneally). Timelines indicate 1 minute intervals. *B*, *C*, Representative traces of 2 rats that received 1 mg cocaine esterase (intravenously) 1 minute after 560 mg/kg WIN-35,065-2 (intraperitoneally). Timelines indicate 2-minute intervals in *B* and 1-minute intervals *C*. The single asterisk indicates the WIN-35,065-2 injection (intraperitoneally) that produced an electrical artifact as the rat was removed from the receiver, interrupting the telemetry signal. The PBS or cocaine esterase injection is identified with double asterisks.

Figure 6.

The effects of cocaine esterase administered after cocaine-induced convulsion (180 mg/kg, intraperitoneally). *A*, A representative trace of a rat that received 0.1 mL PBS (intravenously) immediately after the cocaine-induced convulsion ended. Timelines indicate 30-second intervals. *B*, A representative trace of a rat that received 1 mg cocaine esterase (intravenously) immediately after cocaine-induced convulsion. Timelines indicate 1-minute intervals. *C*, A representative trace from a rat receiving 1 mg cocaine esterase 1 minute after the termination of the cocaine-induced convulsion. Timelines indicate 2-minute intervals. The single asterisk indicates the cocaine injection (intraperitoneally) that produced an electrical artifact as the rat was removed from the receiver, interrupting the telemetry signal. The PBS or cocaine esterase injection is identified with double asterisks.

Figure 7.

A, The effects of the benzodiazepine midazolam on cocaine-induced convulsions and lethality in male rats. Vehicle, 0.32, 1.0, or 3.2 mg/kg midazolam (subcutaneously) was administered as a 15-minute pretreatment to 180 mg/kg cocaine (intraperitoneally). Convulsions and lethality were the observational endpoints, and the percentage of rats affected in each treatment group was recorded. *B*, The effects of midazolam pretreatment on EEG changes produced by cocaine. The baseline amplitude for all traces is approximately 100 μ V. A representative trace from a rat that received 1.0 mg/kg midazolam (subcutaneously) 15 minutes before 180 mg/kg cocaine (intraperitoneally). Timelines indicate 5-minute intervals. The single asterisk and the double asterisks indicate the time of

the cocaine and midazolam injections, respectively. Injections produced an electrical artifact (or interruption in the telemetry signal) because the rat was removed from the receiver. *C*, Expanded trace of the 10 seconds before midazolam administration. *D*, Expanded trace of the 10 seconds before cocaine administration. *E*, A 10-second EEG trace occurring exactly 10 minutes after cocaine injection. *F*, A 10-second EEG trace occurring exactly 1 minute before death, as determined by lack of heartbeat.

Table 1

Characteristics of the convulsions and lethality produced by cocaine and WIN-35,065-2.

Treatment	No. of Rats Convulsing	Average No. of Convulsions per Rat	Onset to Convulsion (s)	Conv Duration (s)	No. of Rats Died	Time to Death (s)
180 mg/kg Cocaine	6/6	1	96 (75–123)	17 (14–20)	6/6	188 (123–266)
560 mg/kg WIN-35,065-2	6/6	4.7 (1–14)	135 (16–240)	21 (16.4–24)	6/6	556 (42–1508)

Table 2

The effects of CocE on convulsions and lethality produced by cocaine or WIN-35,065-2.

Drug	Time Administered	Convulsions	Lethality
Cocaine			
+PBS	1 min after cocaine	6/6	6/6
+CocE	1 min after cocaine	0/6	0/6
Difference, %		100	100
+PBS	Immediately after convulsion	6/6	6/6
+CocE	Immediately after convulsion	6/6	0/6
Difference, %		0	100
+PBS	1 min after convulsion	6/6	6/6
+CocE	1 min after convulsion	6/6	2/6
Difference, %		0	67
WIN-35,065-2			
+PBS	1 min after cocaine	6/6	6/6
+CocE	1 min after cocaine	6/6	6/6
Difference, %		0	0

PBS, Phosphate-buffered saline solution.

In each condition, 6 rats were given either cocaine (180 mg/kg) or the cocaine analog WIN-35,065-2 (560 mg/kg) followed by PBS or CocE administered at times indicated in the table. The *P* value for differences of 100% is .002.