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Effects of cocaine esterase following its repeated administration with cocaine in mice

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Abstract

Background—A bacterial cocaine esterase (CocE) produces robust protection and reversal of cocaine toxicity. The aim of this study was to investigate how effectiveness of CocE was changed following its repeated administration together with cocaine.

Methods—Cocaine toxicity was quantified by measuring the occurrence of convulsions and lethality in mice. Immunologic responses of CocE were determined using ELISA. In the protection experiment, i.v. CocE 0.3 mg was given 1 min before a lethal dose of i.p. cocaine 180 mg/kg. In the rescue experiment, i.v. CocE 0.3 mg was given 1 min after the occurrence of convulsions elicited by i.p. cocaine 100 mg/kg. In both treatment paradigms, 4 trials were conducted in the same animals with a 2-week interval.

Results—CocE retained its effectiveness to protect or rescue mice during the first two trials and these mice did not show an immune response. In contrast, CocE's effectiveness was gradually reduced in the last two trials, accompanied by 10- and 100-fold increases in the anti-CocE antibody titers. Nevertheless, effectiveness of CocE could be partially recovered by increasing the dose of CocE. In addition, escalating the dose of CocE from the minimum effective dose for repeated administration could also retain CocE's effectiveness longer and slow the production of anti-CocE antibodies.

Conclusions—These results indicate that CocE is a weak antigen and it can maintain its protective and rescuing ability initially against cocaine-induced toxicity. Decreased effectiveness of CocE following repeated use can be partially improved by adjusting the dose and frequency of CocE treatment.

Keywords

cocaine esterase; cocaine toxicity; convulsions; lethality; immunogenicity

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1. Introduction

Cocaine abuse continues to be a serious public health problem in the United States (National Institute on Drug Abuse, 2004). Because cocaine abuse is prevalent, cocaine overdose is an issue of concern in emergency departments (Substance Abuse and Mental Health Services Administration, 2006; Devlin and Henry, 2008). Cocaine's local anesthetic effects and monoamine reuptake inhibition can lead to myocardial infarction, cardiac arrhythmias, stroke, and increased myocardial oxygen demand, all of which could lead to fatal consequences (Benowitz, 1993; Egred and Davis, 2005; Afonso et al., 2007). Unfortunately, there is no effective treatment for cocaine toxicity, and the search for effective and safe treatment continues (Dickerson and Janda, 2005; Sofuoglu and Kosten, 2005; Vocci et al., 2005). Given the difficulty in targeting different receptor sites that correspond to the multiple sites of action of cocaine, the protein-based pharmacokinetics may be an alternative therapeutic for cocaine toxicity (Gorelick et al., 2008).

One approach to prevent or alleviate cocaine toxicity is to eliminate it quickly by administration of esterases that rapidly metabolize cocaine. Cocaine esterase (CocE) is the most efficient protein catalyst for the hydrolysis of cocaine studied *in vivo* to date (Larsen et al., 2002; Cooper et al., 2006). CocE was originally identified as a product of the bacterium *Rhodococcus* sp. strain MB1 which grows in the rhizosphere soil of the coca plants in South America. The bacterium uses cocaine as its sole source of carbon and nitrogen by synthesizing CocE to initiate metabolism of cocaine (Bresler et al., 2000). The hydrolytic rate constant (k_{cat}/K_m) of CocE is approximately 1000-fold higher than that of butyrylcholinesterase (BChE), the major cocaine-metabolizing enzyme in the plasma of humans (Inaba et al., 1978; Larsen et al., 2002; Turner et al., 2002). In supporting CocE's superior catalytic efficiency for cocaine, a recent study has demonstrated that i.v. CocE 1 mg protected all rats from i.p. cocaine 180 mg/kg-induced lethality, but i.v. BChE 13 mg (i.e., a 10-fold multiple of the molar equivalent dose of CocE) failed to protect rats from cocaine lethality (Cooper et al., 2006).

More importantly, another study demonstrated that CocE given *after* the occurrence of convulsions not only shortened the duration of convulsions but also saved mice from subsequent death, indicating its therapeutic potential in acute cocaine toxicity after the induction of convulsions (Ko et al., 2007). Nevertheless, CocE is a large, bacterial protein, and as such is liable to elicit an immune response. Although CocE is a weak antigen and repeated exposures of CocE alone may increase its immunogenicity and partially reduce its protective ability (Ko et al., 2007), there is no study conducted to investigate how effectiveness of CocE as a protection or rescue treatment (i.e., before or after cocaine administration) is changed following repeated administration. It is important to elucidate the relationship between effectiveness of CocE and development of anti-CocE antibody. Therefore, the aim of the study was to investigate how the effectiveness of CocE was changed following its repeated administration together with cocaine and whether increasing doses of CocE could counteract with anti-CocE antibodies and retain CocE's protective and rescue ability.

2. Methods

2.1. Subjects

Male NIH-Swiss mice (25–30 g) were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN) and were housed in groups of 4–6 mice per cage. The whole group of mice in one cage was used for the same dosing condition. All mice were allowed *ad libitum* access to food and water, and were maintained on a 12-h light-dark cycle with lights on at 6:30 AM in a room kept at a temperature of 21–22°C. Experiments were performed in the same colony room in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The experimental protocols were

approved by the University Committee on the Use and Care of Animals at the University of Michigan.

2.2. Procedures

2.2.1. Behavioral Assays—Cocaine-induced toxicity was characterized by the occurrence of convulsions and lethality. Cocaine-induced convulsions were defined as loss of righting posture for at least 5 s with the simultaneous presence of clonic limb movements (Ko et al., 2007). Lethality was defined as cessation of observed movement and respiration. Following i.p. cocaine administration, mice were immediately placed individually in Plexiglas containers (16×28×20 cm high) for observation. The presence or absence of convulsions and lethality and the time to affected responses were recorded for 60 min after intraperitoneal (i.p.) administration of cocaine.

2.2.2. Intravenous Administration—The mouse was placed in a small restraint chamber (Outer tube diameter: 30 mm, Inner tube diameter: 24 mm, Model #BS4-34-0012, Harvard Apparatus, Inc., Holliston, MA) that left the tail exposed. The tail was cleansed with an alcohol wipe and a 30G1/2 precision glide needle (Fisher Scientific, Pittsburgh, PA) was inserted into one of the side veins for infusion. The intravenous (i.v.) injection volume of CocE was 0.2 mL per mouse. Sterile gauze and pressure were applied to the injection site to staunch the bleeding.

2.2.3. Serum Collection—Mice have a large vein draining the eye and submandibular area which meet at the rear of the cheek pouch. This vein provides a convenient and consistent source of blood (i.e., cheek-pouch blood sampling). A mouse bleeding lancet, (GoldenRod 4.0 mm animal lancet, MEDIpont Inc., Mineola, NY), was used to puncture this submandibular vein. Each blood sample (200 μ L/mouse) was collected into a BD 400 μ L microtainer tube (Fisher Scientific, Pittsburgh, PA) without preservatives and placed on ice for 60 min. Then, blood samples were centrifuged at 4500 rpm for 5 min at 4 °C. The serum sample (50 μ L/mouse) was pipetted into 2-ml cryovials (Corning Costar Co., Cambridge, MA) and stored at –80 °C until assayed for anti-CocE antibody titer determinations. As soon as the blood was collected, sterile gauze and pressure were applied at the puncture site to minimize the bleeding, and the mouse was returned to its home cage.

2.2.4. Immunological Determination—A direct ELISA specific for anti-CocE antibodies was set up using a standard protocol. CocE was used (1 μ g/mL) to coat a 96-well micro-titer plate using borate buffered saline (1.5 M NaCl, 0.5 M H₃BO₃, 1.0 M NaOH) to resuspend CocE (50 μ L/well). The coating plates were left overnight at 4°C. The coating buffer was removed the following morning and the plates blocked with 2% normal goat serum in phosphate buffered saline for 1 h at 37°C and washed 3 times. Serum from the various groups of mice was serially diluted in 50 μ L of phosphate buffered saline in the wells in a range of 10² to 10⁷ and run in duplicate. The plates were covered and incubated for 1 h at 37°C. Subsequently, the plates were washed 3 times and 50 μ L/well of goat anti-mouse IgG peroxidase labeled antibody diluted 1:400. The plates were then washed 3 times and 100 μ L peroxidase substrate solution (OPD dissolved in citrate/phosphate buffer) was added to each well. After a 5–10 min incubation (based upon color development in the positive controls), the reaction was stopped using 3M H₂SO₄ (50 μ L/well). The plates were read at 490 nm and titer was determined by the highest dilution that showed increases over background absorbance.

2.3. Experimental Designs

The first part of the study was to determine the effectiveness of CocE to protect or reverse cocaine-induced lethality after repeated administration of CocE together with cocaine. In the protection study, CocE 0.3 mg was administered intravenously 1 min before i.p. administration of cocaine 180 mg/kg, which is an LD₁₀₀ of cocaine in mice (Ko et al., 2007). This dosing

condition was repeated every two weeks for 4 times. In the rescue study, CocE 0.3 mg was administered intravenously within the 1st min after the occurrence of convulsions elicited by i.p. cocaine 100 mg/kg. This cocaine dose was chosen because it produced convulsions in 100% of mice and lethality in 50–70% of mice in our pilot study. This dosing condition was repeated every two weeks for 4 times. In both treatment paradigms, anti-CocE antibodies were determined from serum samples collected 24 h before each toxicity test. The toxicity test was performed every 2 weeks because this time interval has been shown to elicit large increases of anti-CocE antibody titers within a short time period of 4–6 weeks (Ko et al., 2007). Therefore, the effectiveness of CocE and the production of anti-CocE antibodies were studied under this specific context with frequent repeated dosing conditions.

The second part of the study was to determine whether two prior exposures of CocE with cocaine change the dose response curves of cocaine for convulsions and lethality. As noted, the early findings from the first part of this study showed that the repeated dosing regimen produced a 10-fold increase in the anti-CocE antibody titers in mice to be tested in the 3rd trial and CocE did not completely retain its ability to protect mice from cocaine toxicity (i.e., 20% of lethality). Such findings are different from previous findings indicating that CocE can fully retain its ability to protect mice against cocaine toxicity (i.e., 0% of lethality) even in the presence of a 10-fold increase of anti-CocE antibody titers (Ko et al., 2007). Thus, the second part of the study was initiated in order to determine whether the susceptibility of mice to cocaine-induced convulsions and lethality was changed after mice had received two prior exposures of CocE with cocaine. After the first two trials in the same dosing regimen used in the first part of the study, this group of mice was used to determine dose response curves (56, 75, 100, and 130 mg/kg, i.p.) of cocaine-induced convulsions and lethality 2 weeks after the 2nd exposure. In addition, a control group of mice was used for comparison under the same condition except they received sham injections of vehicles at the corresponding time points.

The third part of the study was to compare the effectiveness of CocE to protect and reverse cocaine-induced lethality after repeated administration of CocE and cocaine under different escalating dosing regimens. In the protection study, there were three groups and each group was tested under a specific dosing condition. Each dosing condition was repeated every two weeks for 4 times. The first group received the same dose of CocE (0.3 mg) 1 min before i.p. cocaine 180 mg/kg throughout 4 trials. The second group received the same dose of CocE (0.3 mg) for the first 3 trials and a triple dose of CocE (i.e., 0.9 mg) for the 4th trial. The third group received escalating doses, 0.1, 0.2, 0.4, and 0.8 mg, throughout 4 trials (i.e., a 2-fold increase of the dose for each trial). In the rescue study, same dosing regimens for CocE were tested in 3 groups of mice, except CocE was administered intravenously within the 1st min after the i.p. cocaine (100 mg/kg)-induced convulsions. In both protection and rescue studies, anti-CocE antibodies were determined from serum samples collected 24 h before each toxicity test.

2.4. Data analysis

Data from the behavioral toxicity studies (i.e., % of mice showing affected responses) were analyzed with Chi-square probability test with one tail. The values of ED_{100conv} and LD₁₀₀ were determined when 100% of mice showed convulsions or death, respectively. These values were used to compare the degree of shifts of cocaine's dose-response curve in subjects exposed to different dosing regimens. In addition, mean values (mean ± S.E.M.) were calculated from individual values for increased titer numbers. These data were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple (*post hoc*) comparisons. The criterion for significance was set at $p < 0.05$.

2.5. Drugs

(–)Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile water and was administered intraperitoneally at a volume of 0.01 mL/g. CocE (purified and supplied by Drs. D. Narasimhan and R.K. Sunahara, see details in Cooper et al., 2006) was diluted to difference concentrations in phosphate buffered saline and administered intravenously at a volume of 0.2 mL/mouse.

3. Results

Figure 1 shows the effects of repeated administration of CocE and cocaine on the effectiveness of CocE in preventing cocaine-induced toxicity. Intravenous pretreatment with CocE 0.3 mg fully protected mice from i.p. cocaine 180 mg/kg-induced lethality during the first two trials. However, the same dose of CocE failed to protect mice because there were 20–30% and 70–80% of mice died during the 3rd and 4th trials, respectively (Figure 1, top panel). In addition, this dosing regimen significantly produced approximately 10- and 100-fold increases in the anti-CocE antibody titers [$F(3,41)=48.9$, $p<0.05$] in mice to be tested in the 3rd and 4th trials, respectively (Figure 1, bottom panel).

Figure 2 shows the effects of repeated administration of cocaine and CocE on the effectiveness of CocE in reversing cocaine-induced toxicity. Intraperitoneal administration of cocaine 100 mg/kg produced convulsion in 100% of mice tested each time. The averaged intervals between cocaine administration and onset of convulsions were similar, ranging between 2.7 and 3.3 min among all trials (data not shown). Intravenous administration of CocE 0.3 mg as a post-treatment saved convulsing mice from subsequent death during the first two trials. However, the same dose of CocE failed to rescue mice because there were 20–30% and 50–60% of mice died during the 3rd and 4th trials, respectively (Figure 2, top panel). In addition, this dosing regimen significantly produced approximately 10- and 100-fold increases in the anti-CocE antibody titers [$F(3,41)=64.4$, $p<0.05$] in mice to be tested in the 3rd and 4th trials, respectively (Figure 2, bottom panel).

Figure 3 illustrates the dose response curves of cocaine-induced convulsions and lethality in mice under different pretreatment conditions. One group received two prior exposures of i.v. CocE 0.3 mg as a protection treatment against i.p. cocaine 180 mg/kg-induced lethality. The other group received two prior exposures of sham injections with vehicle under the same intervals. There was no significant difference regarding dose response curves of both groups for cocaine-induced convulsions and lethality. Both groups had the same doses of cocaine 100 and 130 mg/kg as $ED_{100conv}$ and LD_{100} , respectively (Figure 3, left panels). In addition, there was no difference regarding the intervals between cocaine administration and onset of convulsions or death between groups (Figure 3, right panels). The averaged intervals to the onset of convulsions after i.p. administration of cocaine 100 mg/kg for the vehicle-pretreated group and the CocE/cocaine-pretreated group were 2.7 ± 0.3 and 2.9 ± 0.3 min (mean $\pm$ S.E.M.), respectively. The averaged intervals to the onset of death after i.p. cocaine 130 mg/kg for the vehicle-pretreated group and the CocE/cocaine-pretreated group were 5.4 ± 0.5 and 5.2 ± 0.6 min, respectively.

Figure 4 illustrates the dose response curves of cocaine-induced convulsions and lethality in mice gone through different exposures of cocaine and CocE. One group received two prior exposures of i.v. CocE 0.3 mg as a rescue treatment against i.p. cocaine 100 mg/kg-induced lethality. The other group received two prior exposures of sham injections with vehicle under the same intervals. There was not significant difference regarding dose response curves of both groups for cocaine-induced convulsions and lethality. Both groups had the same doses of cocaine 100 and 130 mg/kg as $ED_{100conv}$ and LD_{100} , respectively (Figure 4, left panels). In addition, there was no difference regarding the interval between cocaine administration and

onset of convulsions or death between groups (Figure 4, right panels). The averaged intervals to the onset of convulsions after i.p. administration of cocaine 100 mg/kg for the vehicle-pretreated group and the cocaine/CocE-pretreated group were 2.6 ± 0.3 and 2.8 ± 0.6 min, respectively. The averaged intervals to the onset of death after i.p. cocaine 130 mg/kg for the vehicle-pretreated group and the CocE/cocaine-pretreated group were 5.4 ± 0.5 and 5.0 ± 0.7 min, respectively.

Figure 5 compares effects of repeated administration of CocE and cocaine under different dosing regimens on the effectiveness of CocE in preventing cocaine toxicity. The first group (i.e., CocE: 0.3-0.3-0.3-0.3 mg) displayed a similar pattern as reported in the first part of the study. In other words, CocE 0.3 mg provided a full protection in the first two trials, but failed to protect mice in the last two trials. In contrast, the second group (i.e., CocE: 0.3-0.3-0.3-0.9 mg) showed similar results like the first group in the first three trials. Nevertheless, a larger dose of CocE 0.9 mg produced more protection during the 4th trial (Figure 5A), even both groups had similar production of increased anti-CocE antibody titers (the first group: $[F(3,52)=93.6, p<0.05]$, the second group: $[F(3,51)=70.1, p<0.05]$; Figure 5B). More interestingly, the third group (i.e., CocE: 0.1-0.2-0.4-0.8 mg) significantly produced more protection in last two trials compared to the first group (Figure 5C). In terms of anti-CocE antibody development, the third group also significantly had less increased anti-CocE antibody titers (the third group: $[F(3,52)=48.5, p<0.05]$; Figure 5D) as compared to the first group.

Figure 6 compares effects of repeated administration of cocaine and CocE under different dosing regimens on the effectiveness of CocE to reverse cocaine toxicity. The first group (i.e., CocE: 0.3-0.3-0.3-0.3 mg) displayed a similar pattern as reported in the first part of the study. In other words, CocE 0.3 mg saved convulsing mice from subsequent lethality in the first two trials, but failed to rescue mice in the last two trials. In contrast, the second group (i.e., CocE: 0.3-0.3-0.3-0.9 mg) showed similar effects like the first group in the first three trials. Nevertheless, a larger dose of CocE 0.9 mg produced more rescuing effects in the 4th trial (Figure 6A), even both groups had similar production of increased anti-CocE antibody titers (the first group: $[F(3,53)=89.1, p<0.05]$, the second group: $[F(3,53)=95.0, p<0.05]$; Figure 6B). More interestingly, the third group (i.e., CocE: 0.1-0.2-0.4-0.8 mg) significantly produced more rescuing effects in last two trials as compared to the first group (Figure 6C). In terms of anti-CocE antibody development, the third group also significantly had less increased anti-CocE antibody titers (the third group: $[F(3,52)=52.2, p<0.05]$; Figure 6D) compared to the first group.

4. Discussion

This study showed that i.v. administration of CocE 0.3 mg before and after cocaine can either protect or reverse cocaine-induced toxicity in mice. A previous study has shown that i.v. pretreatment with CocE, 0.1 – 1 mg, dose-dependently protected mice from cocaine-induced lethality. More importantly, the same doses of CocE were effective as a rescue agent after the occurrence of convulsion in shortening the duration of convulsions and saving mice from subsequent death (Ko et al., 2007). Such findings agree with the prediction from *in vitro* studies (Larsen et al., 2002; Turner et al., 2002) and further support CocE's therapeutic potential for acute cocaine toxicity by demonstrating CocE's robust protection and reversal of cocaine toxicity *in vivo* (Cooper et al., 2006; Ko et al., 2007).

Interestingly, CocE's effectiveness was gradually decreased following repeated administration (Figures 1 and 2). In particular, the degree of reduced effectiveness as protection or rescue treatment is associated with the development of increased anti-CocE antibody titers. These repeated test sessions were conducted with a 2-week interval. It should be noted that a single prior exposure of CocE did not significantly elicit an immune response, and three prior exposures of CocE weekly only slightly increased the anti-CocE antibody titers (Ko et al.,

2007). These findings indicate that CocE may be a weak antigen and its effectiveness may be partially reduced only after extensive repeated exposures. More importantly, CocE's low risk of immunogenicity does not greatly affect its ability to rescue patients who first time need an immediate treatment for acute cocaine overdose.

A previous study showed that CocE is able to fully retain its effectiveness even in subjects who displayed a 10-fold increase of anti-CocE antibody (Ko et al., 2007). However, the present study shows that CocE did not completely retain its ability to protect all mice from cocaine-induced lethality in the 3rd trial when a 10-fold increase in the anti-CocE antibody titers was detected (Figures 1 and 2). It is possible that repeated exposures of cocaine with CocE especially in the rescue treatment paradigm damage the myocardium and affect the subsequent effectiveness of CocE as a treatment antidote. However, following two prior exposures of cocaine with CocE, mice showed similar dose response curves for cocaine-induced convulsions and lethality as the vehicle-pretreated group did (Figures 3 and 4). These findings indicate that the susceptibility of these cocaine-pretreated mice to cocaine-induced toxicity, measured in both percent of affected subjects and onset of affected responses, is not significantly changed. It may indicate that a partial reduction of CocE's effectiveness in the 3rd trial is mainly due to the increased anti-CocE antibody titers.

To further investigate the relationship between effectiveness of CocE and development of anti-CocE antibodies, the different repeated dosing regimens of CocE with cocaine were conducted. In the 4th trial that two groups of mice had a similar degree of increased anti-CocE antibody titers (Figure 5B), the group received a triple dose of CocE 0.9 mg significantly provided more protection than the group received CocE 0.3 mg (Figure 5A). This finding suggests that increasing the amount of CocE may counteract with the action of anti-CocE antibodies. More importantly, if the dose of CocE used in the first trial starts from the minimum effective dose (i.e., 0.1 mg) and the doses are escalated slowly with a 2-fold increase for each trial, this repeated dosing regimen retains the effectiveness of CocE longer (Figure 5C) and slows the production of anti-CocE antibody (Figure 5D), as compared to the group receiving repeated exposure to the constant dose of CocE 0.3 mg. Similar findings were also found in the rescue treatment paradigm by changing CocE's doses and repeated dosing regimens (Figure 6). These promising results indicate that reduced effectiveness of CocE following repeated administration can be improved by adjusting the starting dose and treatment frequency of CocE.

To date, there is no information in terms of how long the anti-CocE antibody titers are detectable at significant levels once formed. In particular, it is not known whether repeated CocE treatments can be used if a sufficient interval of time such as 6 months or 1 year elapses between treatments. Future studies using endotoxin-free CocE under different repeated dosing intervals (e.g., 1 month vs. 6 months) can further clarify whether this enzyme can be approved by FDA to be used more than one time in humans for cocaine toxicity treatment, or whether one treatment is the maximum ever unless immunosuppressive drugs are used. In addition, pegylated CocE may be another approach to solve the immunogenicity of CocE. Pegylation is the modification of biological molecules by covalent conjugation with polyethylene glycol (Harris and Chess, 2003; Veronese and Mero, 2008). Although pegylation may change the physical and chemical properties of the molecule, pegylated molecules including proteins and peptides have decrease immunogenicity (Harris and Chess, 2003; De Groot and Scott, 2007; Veronese and Mero, 2008). It is important to investigate the effectiveness of pegylated CocE and to compare the degree of immunogenicity of CocE and pegylated CocE following repeated dosing regimens under this context.

In summary, this study demonstrates that CocE produces robust protection and reversal of cocaine toxicity and provides *in vivo* evidence for the therapeutic potential of CocE in the treatment of acute cocaine toxicity. Repeated use of CocE may gradually reduce CocE's

effectiveness as a protection or rescue treatment due to the production of anti-CocE antibodies. It should be reinstated that CocE's weak immunogenicity should not detract its potential value as a treatment antidote for acute cocaine overdose, especially it is intended for patients seeking for treatment first time. Nevertheless, the study provides proof of concept that increasing the doses of CocE can counteract with the action of anti-CocE antibodies and retain a moderate protection or rescue against cocaine toxicity following repeated administration.

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Figure 1.

Effectiveness of CocE in preventing cocaine-induced toxicity is gradually decreased following repeated administration. The protective effects of i.v. CocE (0.3 mg, 1 min pretreatment) against i.p. cocaine 180 mg/kg-induced lethality were tested in the same mice (n=12) every two weeks. The anti-CocE antibody titers were determined from serum samples collected 24 hr *before* the toxicity test. Each value represents the percentage of mice affected (top panel) or the mean $\pm$ SEM (bottom panel). The asterisk represents a significant difference from the value determined in the first trial (*, $p < 0.05$).

Figure 2.

Effectiveness of CocE for interrupting cocaine-induced toxicity is gradually decreased following repeated administration. The rescue effects of i.v. CocE 0.3 mg were studied by administering CocE intravenously within the 1st min *after* the occurrence of convulsions induced by i.p. cocaine 100 mg/kg. This dosing condition was repeated in the same mice (n=12) every two weeks. The anti-CocE antibody titers were determined from serum samples collected 24 hr *before* the toxicity test. Each value represents the percentage of mice affected (top panel) or the mean $\pm$ SEM (bottom panel). The asterisk represents a significant difference from the value determined in the first trial (*, $p < 0.05$).

Figure 3.

No significant change in the dose response curves of cocaine-induced toxicity in mice under different CocE pretreatment conditions. Symbols of filled triangles represent mice which had received two prior exposures of i.v. CocE 0.3 mg as protection treatment against i.p. cocaine 180 mg/kg-induced lethality and were tested for their responses to a single dose of cocaine two weeks after the 2nd exposure. Symbols of open circles represent mice which had received two prior exposures of sham injections of vehicles under the same intervals. Left panels: dose-response curves of cocaine-induced convulsions and lethality. Right panels: time to toxic effects of cocaine in the dosing condition that more than 60% of mice showed affected responses. Each value represents the mean $\pm$ SEM (n=6). See Figure 1 for other details.

Figure 4.

No significant change in the dose response curves of cocaine-induced toxicity in mice gone through different exposures of cocaine and CocE. Symbols of filled triangles represent mice which had received two prior exposures of i.v. CocE 0.3 mg as rescue treatment against i.p. cocaine 100 mg/kg-induced lethality and were tested for their responses to a single dose of cocaine two weeks after the 2nd exposure. Symbols of open circles represent mice which had received two prior exposures of sham injections under the same intervals. Left panels: dose-response curves of cocaine-induced convulsions and lethality. Right panels: time to toxic effects of cocaine in the dosing condition that more than 60% of mice showed affected responses. Each value represents the mean $\pm$ SEM (n=8). See Figure 2 for other details.

Figure 5.

Comparison of CocE's effectiveness in preventing cocaine-induced toxicity following different repeated dosing regimens. The protective effects of i.v. CocE (1 min pretreatment) against i.p. cocaine 180 mg/kg-induced lethality were tested in the same mice ($n=14-15$ per group) every two weeks. The 1st group received the same dose (0.3 mg) of CocE throughout 4 trials (shown in all panels). The 2nd group received the same dose (0.3 mg) of CocE for the first three trials and a larger dose of CocE (0.9 mg) for the fourth trial (panels A & B). The 3rd group received escalating doses of CocE from 0.1 mg in the first trial to 0.8 mg in the fourth trial (panels C & D). The anti-CocE antibody titers were determined from serum samples collected 24 hr *before* the toxicity test. Each value represents the percentage of mice affected (top panels) or the mean $\pm$ SEM (bottom panels). The asterisk represents a significant difference from the value determined in the first trial for each group (*, $p<0.05$). The symbol # indicates a significant difference between groups in the corresponding trial (#, $p<0.05$).

Figure 6.

Comparison of CocE's effectiveness for interrupting cocaine-induced toxicity following different repeated dosing regimens. The rescue effects of i.v. CocE were studied by administering CocE intravenously within the 1st min *after* the occurrence of convulsions induced by i.p. cocaine 100 mg/kg. This dosing condition was repeated in the same mice (n=14–15 per group) every two weeks. The 1st group received the same dose (0.3 mg) of CocE throughout 4 trials (shown in all panels). The 2nd group received the same dose (0.3 mg) of CocE for the first three trials and a larger dose of CocE (0.9 mg) for the fourth trial (panels A & B). The 3rd group received escalating doses of CocE from 0.1 mg in the first trial to 0.8 mg in the fourth trial (panels C & D). The anti-CocE antibody titers were determined from serum samples collected 24 hr *before* the toxicity test. Each value represents the percentage of mice affected (top panels) or the mean $\pm$ SEM (bottom panels). The asterisk represents a significant difference from the value determined in the first trial for each group (*, $p < 0.05$). The symbol # indicates a significant difference between groups in the corresponding trial (#, $p < 0.05$).