A catalytic antibody against cocaine prevents cocaine's reinforcing and toxic effects in rats

BEREND METS*, GAIL WINGER‡, CAMILO CABRERA‡, SUSAN SEO‡, SUBHASH JAMDAR*, GINGER YANG‡, KANG ZHAO‡, RICHARD J. BRISCOE†, ROWENA ALMONTE‡, JAMES H. WOODS†§¶, AND DONALD W. LANDRY‡

*Department of Anesthesiology, Columbia University, College of Physicians & Surgeons, 630 West 168th Street, New York, NY 10032; †Department of Pharmacology, 1301 MSRBIII, University of Michigan Medical School, Ann Arbor, MI 48109-0632; ‡Department of Medicine, Columbia University, College of Physicians & Surgeons, 630 West 168th Street, New York, NY 10032; and §Department of Psychology, 525 East University, University of Michigan, Ann Arbor, MI 48109-1109


ABSTRACT Cocaine addiction and overdose have long defied specific treatment. To provide a new approach, the high-activity catalytic antibody mAb 15A10 was elicited using a transition-state analog for the hydrolysis of cocaine to nontoxic, nonaddictive products. In a model of cocaine overdose, mAb 15A10 protected rats from cocaine-induced seizures and sudden death in a dose-dependent fashion; a noncatalytic anticocaine antibody did not reduce toxicity. Consistent with accelerated catalysis, the hydrolysis product ecgonine methyl ester was increased >10-fold in plasma of rats receiving mAb 15A10 and lethal amounts of cocaine. In a model of cocaine addiction, mAb 15A10 blocked completely the reinforcing effect of cocaine in rats. mAb 15A10 blocked cocaine specifically and did not affect behavior maintained by milk or by the dopamine reuptake inhibitor bupropion. This artificial cocaine esterase is a rationally designed cocaine antagonist and a catalytic antibody with potential for medicinal use.

Cocaine is presently abused in the United States by ~2 million hardcore addicts and >4 million regular users (1). The acute toxicity of cocaine overdose frequently complicates abuse and the potential medical consequences of this syndrome include convulsions and death (1). Despite decades of effort, however, no useful antagonists of cocaine’s reinforcing or toxic effects have been identified. This failure is due, in part, to the drug’s mechanism of action as a competitive blocker of neurotransmitter reuptake (2). Cocaine’s blockade of a dopamine reuptake transporter in the central nervous system is hypothesized to be the basis of its reinforcing effect (3), and the difficulties inherent in blocking a blocker appear to have hindered the development of antagonists for addiction. Further, dopamine appears to play such a general role in many types of behavior that dopamine receptor agonists and antagonists that might be expected to modify cocaine’s actions do not act selectively (4). For cocaine overdose, this problem is compounded by the binding of cocaine at high concentrations to multiple receptors in the central nervous system and the cardiovascular system. For example, blockade of serotonin reuptake transporters contributes to cocaine-induced convulsions (5); dopamine reuptake blockade (5, 6), and dopamine D1 receptor binding (6) contribute to lethality; and blockade of norepinephrine-reuptake transporters, as well as blockade of cardiac myocyte Na+ channels and other ion transporters, contribute to arrhythmias and sudden death (7). Thus, cocaine abuse and toxicity may well pose insurmountable problems for the classical receptor-antagonist approach.

These difficulties in developing antagonists for cocaine led us to embark on an alternative approach—to intercept cocaine with a circulating agent, thereby rendering it unavailable for receptor binding. An antibody is a natural choice for a circulating interceptor, and, in 1974, antiheron antibodies were shown to block heroin-induced reinforcement in a rhesus monkey (8). However, the binding of heroin depleted the neutralizing antibody stoichiometrically and self-administration resumed. Our solution to the limitation imposed by simple binding was to develop catalytic antibodies—the newly discovered class of artificial enzymes (9)—with the capacity to bind and degrade cocaine, release product, and become available for further binding. Cocaine can be effectively degraded by hydrolysis of its benzoyl ester, because the resulting products, ecgonine methyl ester and benzoic acid (Fig. 1A), are neither reinforcing nor toxic (10). To obtain antibodies able to catalyze this reaction, we synthesized a phosphonate monoester transition-state analog for benzoic esterolysis (TSA-I, Fig. 1B) and with this immunogen elicited the first artificial enzymes able to degrade cocaine in vitro (11).

Through repetitive hybridoma preparation with TSA-I, we recently generated the murine mAb 15A10 (12). mAb 15A10 is the most potent artificial cocaine esterase to date with a Michaelis constant of 220 μM, a turnover rate of 2.3 min⁻¹, and a rate acceleration of 2.4 × 10⁴. The antibody retained >95% of its activity after >200 turnovers, and product inhibition, a frequent impediment to useful antibody catalysis (9), was not observed for the alcohol product ecgonine methyl ester at concentrations up to 1 mM. Although mAb 15A10 was inhibited in vitro by benzoic acid (Ka, ~250 μM), this acid is rapidly cleared from plasma through coupling to glycine (13) and the adduct, hippuric acid, was not an inhibitor in vitro at a concentration of 1 mM. Thus, mAb 15A10 possessed several characteristics essential for a catalyst to be used in vivo.

We now describe the first in vivo studies of an anticocaine catalytic antibody. We examined the effect of mAb 15A10 on seizure and lethality in a rat model of overdose and its effect on cocaine-induced reinforcement in a rat model of addiction.

METHODS

Preparation of mAb 15A10. Hybridoma 15A10 was seeded in a Fibra Cell support matrix (Collagen Plus bioreactor, New Brunswick Scientific) continuously perfused with RPMI 1640 (GIBCO) medium. Perfusate was concentrated with a preparative scale 10-kDa cut-off, 6 sq. ft. ultrafiltration cartridge (Millipore) and then subjected to protein G chromatography to yield mAb 15A10 >90% pure by SDS/PAGE chromatography. Catalytic activity was comparable to that previously described (12) and was completely inhibited by free TSA (10)

ABBREVIATION: TSA-I, phosphonate monoester transition-state analog for benzoic esterolysis.

© 1998 by The National Academy of Sciences 0027-8424/5/9510176-62.00/0
PNAS is available online at www.pnas.org.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

1998 by The National Academy of Sciences 0027-8424/5/9510176-62.00/0
PNAS is available online at www.pnas.org.

1998 by The National Academy of Sciences 0027-8424/5/9510176-62.00/0
PNAS is available online at www.pnas.org.
lysis and the corresponding phosphonate monoester analog (50 mg). With a trial pressure being transduced, and saline or antibody (5, 15, or 100 units) solution and then filled with 0.9% NaCl containing 100 mg heparin. Twenty-four hours after cannulation, arterial plasma samples were obtained at death for determination of cocaine and metabolite concentrations using HPLC liquid chromatography (17).

**Rat Self-Administration Protocol.** To evaluate the reinforcing effects of cocaine, five male Sprague–Dawley rats, weighing between 300 and 350 g, were initially trained in operant conditioning chambers (Colbourn Instruments, Lehigh Valley, PA) to press on a lever and receive access to 0.5 cc sweetened condensed milk during daily 1-hr sessions. The operant conditioning chambers were equipped with a stimulus light over the lever that could be illuminated red or green, a white house light, and a retractable lever. At the start of each 1-hr session, the lever was extended into the chamber and the red stimulus light was illuminated. During delivery of the reinforcer (initially milk, and later 0.3 mg/kg per injection of cocaine or 1 mg/kg per injection of bupropion) the red stimulus light was turned off, the green stimulus light was turned on, and the lever remained retracted. The illumination of the red light and the presentation of the lever, along with the absence of the house light signaled that the response contingencies were again effective.

The number of reinforcers necessary to produce 5 sec of milk presentation was increased from 1 to 5 (fixed ratio 5). When the rats were responding and receiving >50 milk reinforcers at an FR 5 for three consecutive days, an intravenous catheter was surgically implanted in each rat (18). A polyurethane catheter (0.6 mm ID × 0.9 mm OD), 13 cm in total length, was inserted into the external jugular vein; the distal end exited between the rats’ shoulder blades where it was fastened to a connector (Plastics One, Roanoke, VA). The catheters were flushed daily with 0.9% NaCl solution containing heparin (100 units/ml), and the animals were allowed five days to recover after surgery. During subsequent sessions, the catheters were connected to an infusion pump (Harvard Apparatus) and the conditioning chambers were changed so that responding resulted in the intravenous delivery of cocaine. The rats were weighed before each session, and the infusion duration was adjusted according to each animal’s weight so that the amount of cocaine (1 mg/ml) delivered with each injection equaled 0.3 mg/kg. The fixed ratio was reduced to 1 at the time cocaine was made available, and increased gradually to 5 over the course of five days. When the rats were receiving at least 20 injections of cocaine per session for three consecutive days, saline replaced the cocaine in the infusion pump for a single session. Saline was substituted on two or three sessions, each separated by single sessions of cocaine availability, until the pattern of saline self-administration or the number of saline injections was clearly different from that observed with cocaine self-administration. The difference in pattern of drug or saline self-administration was summarized with a “half-life” calculation. This was the number of minutes of the 1-hr session that passed at the time 50% of the total number of responses had been made. Thus, if responding occurred at a constant rate during the 60 min session, the half-life would be 30 min.

In the second experiment, Sprague–Dawley rats (350–400 g) housed under identical conditions, were similarly catheterized, and given either mAb 15A10 100 mg/kg (n = 4), mAb 1C1 100 mg/kg (n = 4), or saline (n = 17). The total volume for all treatments was 5 cc given intravenously over 5 min. After 15 min, cocaine was infused intravenously at a rate of 1 mg/kg/min with catecholamines as above until cardiopulmonary arrest. Arterial plasma samples were obtained at death for the corresponding phosphonate monoester analog (50 mg). With a trial pressure being transduced, and saline or antibody (5, 15, or 100 units) solution and then filled with 0.9% NaCl containing 100 mg heparin. Twenty-four hours after cannulation, arterial plasma samples were obtained at death for determination of cocaine and metabolite concentrations using HPLC liquid chromatography (17).

**Preparation and Characterization of mAb 1C1.** mAb 1C1 was obtained from the original hybridoma preparation with TSA-I as described (12) and was chosen as a noncatalytic control because its binding affinity for cocaine was superior to that of mAb 15A10. For mAb 1C1, the cocaine IC50 was 30 μM by inhibition of 3H-cocaine binding (31 μCi/ml, New England Nuclear) with cold cocaine 0–1,000 μM in phosphate buffered saline (pH 7.4). Bound radiolabel was separated from free by gel filtration chromatography using a Sephadex G-50 (2 ml) spin column (2,000 g × 30 sec).

**Cocaine Toxicity in the Rat.** We utilized our previously reported model for cocaine toxicity based on coinfusion of catecholamines (14). The toxicity of cocaine can vary significantly among individuals depending on endogenous catecholamine levels, and this likely explains the variably increased incidence of sudden death in restrained animals (15) and agitated patients (16). In previous work (14) we standardized catecholamine levels through intravenous infusion in conscious, unrestrained animals and, for continuously infused cocaine (1 mg/kg/min), found that the LD50 was 10 mg/kg and the LD90 was 16 mg/kg. Using this model, two experiments were aimed at determining the protective effects of mAb 15A10 against cocaine toxicity. In the first, 18 male Sprague–Dawley rats (350–400 g) were housed in standard polycarbonate cages (10.5” × 19” × 8”) on corn cob bedding (Fisher), and given ad libitum Rodent Chow (Fisher) and water. The animals were kept on a 12-hr light-dark cycle. They were fitted with femoral arterial and venous catheters under pentobarbital anesthesia. The catheters were flushed with an 0.9% NaCl solution and then filled with 0.9% NaCl containing 100 units/ml heparin. Twenty-four hours after cannulation, arterial pressure was transduced, and saline or antibody (5, 15, or 50 mg/kg) was administered followed after 15 min by coinfusion of cocaine (1 mg/kg/min) and catecholamines [norepinephrine (0.725 μg/kg/min), epinephrine (0.44 μg/kg/min), and dopamine (0.8 μg/kg/min)]. The infusion was continued for 16 min to deliver the LD90 of cocaine, unless the animals expired earlier.
earning milk reinforcers, a 2 min timeout was added after each milk delivery so that the total number of milk reinforcers earned was similar to the number of cocaine reinforcers earned. The four animals earning bupropion reinforcers (1 mg/kg per injection) had a schedule of drug delivery identical to that for cocaine.

The antibody was concentrated by ultrafilter-centrifugation and the rats received 9–12 mg of the antibody intravenously in 1–5 cc. The volume of injected solution varied depending on the activity of the batch of antibody used. The reinforcing effects of cocaine, bupropion, or milk in these rats were evaluated 24 hr after antibody administration.

RESULTS

Cocaine Toxicity in the Rat. Rats pretreated with mAb 15A10 showed a significant (P < 0.001) dose-dependent increase in survival after an LD₉₀ cocaine infusion (Fig. 2). Four of five animals receiving 15 mg/kg antibody, and all of five receiving 50 mg/kg antibody, survived. In contrast, all eight untreated rats expired before the cocaine infusion was complete. In the animals not treated with mAb 15A10, the mean cocaine dose at death was 7.5 ± 0.6 mg/kg, whereas the five treated with antibody at 5 mg/kg expired at a mean cocaine dose of 8.2 ± 1.0 mg/kg and the single nonsurvivor in the group treated with antibody at 15 mg/kg expired at 15.9 mg/kg of cocaine.

To quantify further the protective effect of the catalytic antibody, we infused catecholamines and cocaine continuously to the experimental (mAb 15A10, 100 mg/kg) and control (saline) groups until all animals expired (Fig. 3). The dose of cocaine at seizure averaged 9.48 mg/kg for saline controls and 32.5 mg/kg for animals treated with mAb 15A10 (P < 0.01). The mean lethal dose of cocaine was also increased 3-fold, from 11.5 mg/kg of cocaine for controls to 37.0 mg/kg for the mAb 15A10 group (P < 0.01).

Simple binding was an unlikely explanation for the effectiveness of mAb 15A10 because stoichiometric binding of...
cocaine would be expected to shift the dose-response to cocaine by <1 mg/kg. However, to exclude this possibility, we tested the action of a binding antibody, mAb 1C1, at the same dose. mAb 1C1 was elicited by immunization with TSA-I but the antibody is not catalytically active because it binds free TSA and cocaine with comparable affinity. As expected mAb 1C1, despite its greater affinity for cocaine compared with mAb 15A10, was ineffective in blocking cocaine-induced convulsions or death (Fig. 3A).

To demonstrate in vivo catalysis, we measured the plasma concentrations of cocaine hydrolysis products in the 15A10 and control groups by our previously developed HPLC method (17). The mAb 15A10 group showed a >10-fold increase in ecgonine methyl ester compared with either the saline (P < 0.001) or the mAb 1C1 (P < 0.01) control groups (Fig. 3B). As expected, based on its rapid metabolism (13), plasma benzoic acid concentrations were not significantly elevated in the 15A10 group (3.85 ± 0.89 μM) compared with the saline control group (2.36 ± 1.05 μM) [data not shown]. Consistent with specific catalysis at the benzoyl ester, the plasma concentration of the methyl ester hydrolysis product, benzoyl ecgonine, was not significantly increased in the mAb 15A10 group (7.68 ± 1.07 μM) compared with saline control (5.47 ± 1.01 μM) [data not shown].

We measured plasma cocaine concentrations at death using HPLC (17) in control rats and those receiving mAb 15A10 to confirm that mAb 15A10 conferred resistance to cocaine toxicity through a prereceptor mechanism. A marked elevation of plasma cocaine at death would be expected if mAb 15A10 blocked toxicity by acting at or after the binding of cocaine to its receptors. In contrast, plasma cocaine concentrations at death were not significantly different between 15A10 and control groups (Fig. 3B) despite the administration of >3-fold higher dose of cocaine to the mAb 15A10 group (Fig. 3A). This result for mAb 15A10 was as expected for a prereceptor effect and consistent with protection from toxicity through catalyzed degradation of cocaine.

**Rat Self-Administration.** In this evaluation, the rats were prepared with chronic indwelling intravenous catheters and trained to press on levers and receive intravenous injections of 0.3 mg/kg/inj cocaine during 1-hr daily sessions. Cocaine maintained regular patterns of lever-pressing; when saline was substituted for cocaine, lever-pressing decreased rapidly during a session (Fig. 4A and B). mAb 15A10 blocked completely the reinforcing effects of intravenous cocaine in the rat; both half-life value was 12.7 min and the average number of injections was 11.6 (Fig. 5). These values were significantly different from those for cocaine in the absence of antibody administration [ANOVA, F(2, 12) = 27.1, P < 0.001; Tukey post hoc test, P < 0.001].

![Fig. 5. Comparison of the number of injections and response half-life (time required for 50% of the total injections to be taken) for responding maintained by cocaine, saline, or cocaine after administration of mAb 15A10. An asterisk indicates a significant difference from cocaine (P < 0.001; Tukey post hoc test).](image)

![Fig. 6. Comparison of the response half-lives for milk reinforcer and milk reinforcer after administration of mAb 15A10 (A) and for responding maintained by bupropion, saline, or bupropion after administration of mAb 15A10 (B). ANOVA indicated a significant difference [F(2, 9) = 70.28, P < 0.00001]. An asterisk indicates a significant difference from saline (P < 0.0001; Tukey post hoc test).](image)
catalyzed by a dopamine antagonist-based treatment of cocaine abuse.

The possibility remained that the action of mAb 15A10 was due to a nonspecific effect on the dopaminergic reward pathway. To test this possibility, we evaluated the ability of 9–12 mg mAb 15A10 to modify self-administration of the dopamine reuptake inhibitor bupropion. This stimulant maintained a pattern of responding that was much like that maintained by cocaine, and substitution of saline led to a rapid change in patterns of responding. In contrast, administration of mAb 15A10 did not alter the pattern (Fig. 6D) or amount of bupropion self-administered by the rats.

In summary, the catalytic antibody was extremely selective in blocking behavior maintained by cocaine. Because catalytic antibodies act outside the central nervous system, this approach to cocaine pharmacotherapy will not be complicated by the cocaine-like side effects expected with agonist-based treatments, or the neuroleptic side effects as might be expected with a dopamine antagonist-based treatment of cocaine abuse.

DISCUSSION

These data demonstrate that a catalytic antibody raised to a transition-state analog of cocaine hydrolysis is able to increase the rate of cocaine degradation in vivo, protect against cocaine’s lethal effects, and block its reinforcing effects. The potential usefulness of an effective anticocaine therapeutic can be inferred from two decades of experience with the pharmacologic treatment of heroin addiction. Heroin treatment programs that employ both counseling and methadone report abstinence rates between 60% and 80%, in contrast to 10% and 30% for programs that rely strictly on nonpharmacological approaches. In contrast to methadone, which is itself addictive, a catalytic antibody would not be expected to enter the brain and therefore would not be psychoactive. Whereas simple blockers of heroin such as naltrexone are useful for treating withdrawal and therefore would not be psychoactive, catalytic antibodies are likely to be longer-lived in plasma than natural enzymes and, in contrast to typical antibodies that can form practically irreversible complexes with antigen, catalytic antibodies are not susceptible to depletion by the act of complex formation with cocaine. Thus, catalytic antibodies have the unique potential to treat both the acute and chronic aspects of cocaine abuse.

Based on a simple compartment model, we previously estimated that an antibody with a turnover rate ($k_{cat}$) of $\approx 2 \text{ sec}^{-1}$ would be needed to commence clinical trials (11). However, this model neglected the large volume of distribution of cocaine and therefore our estimate provided only an upper limit on the activity required. The recent demonstration that high affinity antibodies with no catalytic activity can interfere with cocaine-induced reinforcement in rats (21) suggests that the parameters for a therapeutically effective catalytic antibody will be markedly lower than $2 \text{ sec}^{-1}$. Thus, a humanized version of mAb 15A10 with little to no improvement in turnover rate ($k_{cat} = 2.3 \text{ min}^{-1}$) but with increased affinity for cocaine ($K_m = 1–10 \mu M$) could be therapeutically useful. Protein engineering to humanize mAb 15A10 and random mutagenesis to optimize $K_m$ and $k_{cat}$ are in progress and a panel of catalytic antibodies of varying $K_m$ and $k_{cat}$ will allow us to explore the relationship of binding and turnover to clinical efficacy.

Discussions with Q. Al-Awqati, M. Fischman, and H. Kleber and the technical assistance of Merilee Olsen and Sarah Sommers are gratefully acknowledged. This work was supported by the Counterdrug Technology Assessment Center at the Office of National Drug Control Policy (D.W.L.) and by Public Health Service Grants DA-00245 (J.H.W.) and DA-09163 (G.W.). Postdoctoral support included an Aaron Diamond Research Fellowship (G.Y.) and Substance Abuse (DA-07268) (R.J.B.). Cocaine was supplied by the National Institute on Drug Abuse, Rockville, MD; bupropion was a gift from Glaxo–Wellcome, Research Triangle Park, NC. Experiments were performed with prior approval of Institutional Animal Care and Use Committees.