Natural and Artificial Enzymes Against Cocaine. I. Monoclonal Antibody 15A10 and the Reinforcing Effects of Cocaine in Rats

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ABSTRACT

Recent reports have indicated the potential usefulness of anti-cocaine catalytic monoclonal antibodies in reducing cocaine's toxic and reinforcing effects by altering its pharmacokinetics to favor increased metabolism to the systemically inert products egressive methylester and benzoic acid. The present study was designed to further these findings by evaluating the hypothesis that administration of the anticocaine catalytic monoclonal antibody mAb 15A10 would dose and time dependently reduce behavior maintained by a range of doses of i.v. cocaine. Male Sprague-Dawley rats were trained in daily 8-h sessions to self-administer i.v. cocaine. A within-session multiple-dose protocol was used wherein rats were allowed access to saline or one of six doses of cocaine [0 (saline), 0.015, 0.03, 0.06, 0 (saline), 0.125, 0.25, or 0.5 mg/kg/injection] each hour in the order stated. After demonstrating stable dose-response curves over 3 consecutive days, rats were given 30-min pretreatments of saline or mAb 15A10, (10, 30, or 100 mg/kg i.v.). Antibody, but not saline, pretreatments significantly altered dose-response curves for cocaine self-administration in a dose- and time-dependent manner, resulting in downward and rightward shifts in rates of responding across the cocaine dose range. These effects were apparently not attributable to general behavioral suppression, because operant behavior for an alternative reinforcer was not likewise affected. The present data extend previous work indicating that pharmacokinetic approaches may be of worth in the search for clinically effective cocaine antagonists.

Cocaine abuse continues to present a significant medical and social problem in modern societies. In the search for effective pharmacotherapies to aid in the treatment of cocaine abuse, a variety of drugs have shown promise in preclinical screens. Many proposed treatments for cocaine abuse have been targeted toward either simulating or blocking cocaine's actions within the central nervous system, most notably with respect to its effects on dopaminergic transporters and receptors (Rothman and Glowa, 1995; Mello and Negus, 1996; Gatley et al., 1998; Carroll et al., 1999; Pilla et al., 1999). This kind of approach to the problem may generally be described as “pharmacodynamic” in nature, because most such potential treatments have been designed either to mimic or to interfere with the neuromolecular interactions that presumably underlie cocaine's central effects. It is clear that whereas progress in the development of effective treat-ments for cocaine abuse through such an approach is contingent upon the completeness of our understanding of the relevant mechanisms by which cocaine's subjective and reinforcing effects are mediated, we still have much to learn (Rothman and Glowa, 1995).

Preclinical evaluations of agents that act as direct or indirect agonists or antagonists in monoaminergic brain pathways have suggested that these agents may be useful if applied to the problems of human cocaine abuse (Rothman and Glowa, 1995; Mello and Negus, 1996; Carroll et al., 1999). In addition, drugs working through alternate neural systems, such as γ-aminobutyric acid agonists (Roberts and Andrews, 1997; Brebner et al., 1999), also may be effective in blocking various behavioral and toxic effects of cocaine. Nevertheless, none of these have yet been proven to be safe and effective in blocking the subjective and reinforcing effects of cocaine without untoward side effects in well controlled human clinical trials (Warner et al., 1997; Kranzler et al., 1999). The reason for these failures is probably multifactorial, but the most often cited difficulties with pharmacodynamically based therapies are 1) the general finding that cocaine has multiple sites of action within the central ner-

ABBREVIATIONS: mAb, monoclonal antibody; FR, fixed ratio.

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vous system that are not all amenable to interference or blockade with any one particular pharmacologic agent and 2) the tendency of agonist or antagonist therapies to produce undesirable central effects of their own, conceivably leading either to potential abuse of the intended pharmacotherapeutic agent or to noncompliance on the part of the cocaine abuser related to adverse side effects.

Several recent reports (Bagasra et al., 1992; Landry et al., 1993; Carrera et al., 1995, 2000; Yang et al., 1996; Ettinger et al., 1997; Fox, 1997; Mets et al., 1998) have described a different kind of approach to the problem that relies on altering cocaine's pharmacokinetic profile so that none, or a relatively small proportion, of an administered dose crosses the blood-brain barrier to reach the central nervous system, where the drug's subjective and reinforcing effects are realized through a variety of neurochemical systems. According to this concept of pharmacokinetic intervention, antagonism of cocaine's effects may be achieved through the sequestration and/or metabolic conversion of cocaine molecules in the peripheral circulation by systemic administration (passive immunization) of agents such as catalytic monoclonal antibodies, for example, mAb 15A10, previously synthesized and described by Yang et al. (1996).

A recently developed anticocaine catalytic antibody, mAb 15A10, is unique in that it has fair in vitro catalytic activity ($k_{\text{cat}} = 2.3 \text{ min}^{-1}$) in combination with good selectivity and affinity ($K_m = 220 \mu M$) for cocaine (Yang et al., 1996). The effectiveness of mAb 15A10 in antagonizing the systemic toxicity induced by an overdose of cocaine near the LD$_{50}$ has recently been demonstrated in rodents (Mets et al., 1998). Additionally, these authors described an experiment establishing the capacity of 30 to 40 mg/kg i.v. pretreatments of mAb 15A10 to selectively block the reinforcing effects of cocaine (0.3 mg/kg/injection), but not those of loperamide or food. Although this previous work indicated that mAb 15A10 might effectively reduce cocaine-maintained operant responding, the reliability of these effects has not been assessed across a range of doses of cocaine that support behavior or over a range of differing doses of antibody.

The present experiments were conducted to characterize more fully the dose-response profile of mAb 15A10 in terms of its capacity to alter cocaine-reinforced behavior. Several experiments were designed to determine the effects of three different pretreatment doses of mAb 15A10 across a range of cocaine doses available for self-administration on a multiple-dose schedule. It was hypothesized that concentration-dependence effects of mAb 15A10 would be observed on cocaine dose-response functions, with higher antibody concentrations producing relatively greater suppression of responding at all doses of cocaine. It was further hypothesized that mAb 15A10-induced suppression of cocaine-reinforced responding would be time-dependent, with higher concentrations of antibody producing relatively prolonged suppression of cocaine-maintained responding.

### Materials and Methods

#### Subjects.
Male Sprague-Dawley rats ($N = 19$) weighing between 250 and 275 g upon receipt (Harlan, Indianapolis, IN) were maintained in a temperature and humidity controlled colony room with a 12 h light:12 h dark lighting cycle (lights on at 7:30 AM) over the course of these experiments. Food intake in experimental animals was regulated over the course of early operant training to ensure stability in responding; however, all rats had access to water ad libitum. Animals were maintained throughout the course of these studies in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

#### Apparatus.
Six operant chambers (Med Associates, St. Albans, VT) equipped with house light, retractable lever, dipper mechanism, red, yellow, and green stimulus lights, and a pneumatic syringe pump apparatus (ITTC Life Sciences, Inc., Chicago, IL) for drug delivery were interfaced with an IBM-compatible computer through input and output cards (Med Associates). Each of the chambers was housed within a ventilated, sound-attenuating cubicule (Med Associates). Custom self-administration programs, controlling scheduled contingencies and stimulus arrays within the operant chambers, were written using the Med-PC Medstate Notation version 2.0 programming language for DOS (Med Associates).

#### Preoperative Training.
Rats were trained in daily 1-h sessions by the method of successive approximations to press a lever located within the operant chamber to gain a 5-s access to 0.5 ml of a sweetened milk solution. After the subjects had acquired the lever-press response on a fixed-ratio (FR) 1 schedule of reinforcement, the response requirements were successively increased until rats were responding on an FR5 schedule with a 10-s time-out period after each reinforcer delivery. During the 10-s time-out period, the lever was retracted, the house light and green stimulus lights were extinguished, and a red stimulus light was illuminated to signal that programmed reinforcers were not available. These stimulus conditions also prevailed within each individual dose component of the multiple-dose schedule used for cocaine self-administration sessions conducted in later training and testing. After the rats displayed stable rates of milk-reinforced behavior over 3 consecutive days on this schedule (less than 10% variability in reinforcer deliveries over the 1-h session), i.v. catheters were inserted and rats were given a minimum of 2 days, during which training was not conducted, to recover from surgery.

After completing the above training regimen and meeting the criterion for testing, one group of rats ($n = 4$) received jugular catheters as described below, were given 2 days for recovery, and then were allowed to respond in 1-h operant sessions, as before, for contingent presentation of a milk reinforcer (5-s access to 0.5 ml of sweetened milk) on a daily basis. Once behavior had again stabilized on the schedule according to the same criterion ($\pm 10\%$ variability in responding over 3 consecutive days), rats were given 30-min pre-treatments of saline on one day and 100 mg/kg mAb 15A10 on the following day and allowed to respond for milk in their normal operant session on both occasions. This experiment was included to rule out the possibility that any observed alterations in cocaine-reinforced behavior secondary to antibody treatment were related to a general effect on operant behavior.

#### Surgeries.
Intravenous catheterization surgeries were performed under anesthesia provided by separate injections of ketamine hydrochloride (100 mg/kg i.m.) and xylazine hydrochloride (8 mg/kg i.m.). Anesthesia was considered adequate to commence surgery when each rat demonstrated 1) loss of the righting reflex, 2) no eye blink to digital palpation around the orbit, and 3) no muscular or vocal response to firm tail-pinch. A 2.5-cm incision was made through the skin on the dorsal surface, 0.5 cm posterior to the midscapular level and perpendicular to the rostral-caudal axis of the rat. Another 2.5-cm incision was made ventrally on the area of the neck overlying the right jugular vein parallel to the rostral-caudal axis. After the right jugular vein had been isolated through the ventral incision, the tip of the 15-cm long Micro-Renathane catheter (MRE 040, Braintree Scientific, Braintree, MA) was inserted into the vein a total distance of 32 mm. The catheter was sutured to the vein and anchored to the surrounding tissue at four points. The distal end of the catheter was threaded subcutaneously, around the right foreleg to the dorsal incision point, and attached to a stainless steel (Harvard Scientific, Holliston, MA) anchoring button. The anchoring button was then...
sutured to the musculature and secured in place by suturing a 2- × 3-cm piece of sterile Lars Mesh (Meadow Medicals, Oakland, NJ) over the base of the button to the underlying tissue. Both dorsal and ventral incisions were then closed with suture and cyanoacrylate (Super-Glue).

After surgery, each rat was administered 0.1 ml of saline containing penicillin G sodium (250,000 U/ml) through the jugular catheter, followed by 0.3 ml of a heparin solution (50 U/ml) in saline. When animals resumed training, catheters were flushed with 0.1 ml of heparinized saline (50 U/ml) each day immediately before and at the conclusion of self-administration sessions. Catheter patency was checked, when necessary, by the administration of methohexital sodium (0.1 ml of a 10 mg/ml solution) through the jugular catheter, which caused immediate loss of the righting reflex if the catheter was patent. At the conclusion of the studies, rats were euthanized with an overdose of 100 mg/kg pentobarbital sodium (50 mg/ml) delivered through the jugular catheter. Positive determination of catheter patency and proper placement were done by necropsy after euthanasia in each rat.

**Cocaine Self-Administration.** The multiple-dose schedule used for cocaine self-administration is similar in principle to others that have been developed previously (Gerber and Wise, 1989; Emmett-Oglesby et al., 1993; Caine et al., 1999). On the first operant training session after surgery, rats were allowed to respond, in a 1-h session, on the lever for the simultaneous delivery of both milk (access of 5 s) and cocaine (0.125 mg/kg/injection). On the next day, rats were given access to response-contingent cocaine injections only on a randomized dose schedule that allowed subjects to respond for one of three unit doses of cocaine (0.125, 0.25, or 0.5 mg/kg/injection) in consecutive 50-min sessions interspersed with 10 min time-out periods. During time-out periods, the response lever was retracted, the house light and green stimulus lights were extinguished, and a red stimulus light was illuminated to signal drug nonavailability. The 10-min time-out periods between 50-min cocaine sessions were included as an attempt to reduce carryover effects across cocaine-access periods. The Med-PC program varied the cocaine dose administered within each 50-min access period by varying the number of pulses of the syringe. The total session length was 6 h, and each dose was available twice. For example, one possible dose sequence was 0.125, 0.5, 0.125, 0.25, 0.25, and 0.5 mg/kg/injection.

After demonstrating reliable dose-response curves at these three higher doses, rats were switched to a schedule that included three lower doses of cocaine (0.015, 0.03, and 0.06 mg/kg injection) in addition to the three highest doses. Each of these doses was also available for 50 min, followed by a 10-min time-out period, and these three doses were presented randomly during the first 3 h of the 6-h session. At the end of the first 3 h, a stopcock was switched so that a higher concentration of cocaine was connected to the infusion pump, making the higher three doses of cocaine available during the second 3-h session. After rats had again demonstrated stable dose-response curves on this multiple-dose schedule, they were placed on the final schedule in which these same six doses of cocaine were available each day, but always in ascending order. In addition, two 50-min saline components, with 10-min time-out periods, were included, one at the beginning of the session and one in the 5th h of the session. Thus, the order of cocaine availability was 0 (saline), 0.015, 0.03, 0.06, 0 (saline), 0.125, 0.25, and 0.5 mg/kg/injection over the course of the now 8-h session. At the beginning of these sessions, rats received a “priming” injection equivalent to the dose normally available for self-administration within that component.

Each rat had to meet a criterion for stability in rates of responding in this procedure before receiving a pretreatment of mAb 15A10 or a control volume of saline. When put to statistical test (ANOVA), dose-response curves over 3 consecutive days within a given dose group had to be statistically similar (main time and main dose × time interactions; all P > .05). When this criterion was met, the animal was given an i.v. infusion of saline (10 ml) followed by one of three doses of mAb 15A10 (10, 30, or 100 mg/kg i.v.) mixed with saline to produce a final volume of 10 ml, over a 30-min period. Saline was always administered first, because of the possibility of long-term alterations in the subjects’ self-administration behavior after mAb 15A10 treatment. Thirty minutes after the infusion was completed, rats were placed in the operant chambers, and the 8-h cocaine self-administration session was initiated. All testing was done in the light phase of the light/dark cycle.

**Research Design and Statistical Analyses.** A mixed between- and within-subjects, repeated-measures design was used to characterize the influence of mAb 15A10 on dose-response profiles of cocaine self-administration. Each of the three groups of rats (n = 5 per group) was given either saline or one of three different concentrations of catalytic antibody (10, 30, or 100 mg/kg i.v.) mixed with saline to produce a final volume of 10 ml. The three different doses of antibody constituted the between-subjects factor. The effects of the pretreatments were monitored for several days after treatment until the rats’ self-administration behavior returned to baseline (pretreatment) values. Time (responding on days after either saline or mAb 15A10 infusions in addition to any subsequent days after infusion) and cocaine dose thus represented the within-subjects factors in these experiments. Dose-response curves collected after administration of the three different treatment concentrations of catalytic antibody were analyzed with mixed-factor ANOVA with Geisser-Greenhouse adjustment. Antibody concentration (10, 30, or 100 mg/kg) was loaded as the between-subjects factor, and cocaine dose [0 (saline), 0.015, 0.03, 0.06, 0.125, 0.25, and 0.5 mg/kg/injection] and days (saline versus mAb 15A10 pretreatment) were loaded as within-subjects factors. All individual comparisons across antibody treatment groups were conducted with the Tukey honest significant difference post hoc procedure. Data were further analyzed within each antibody pretreatment group by one-way ANOVA, with cocaine dose and days (saline versus mAb 15A10 pretreatment and all consecutive days of testing) loaded as repeated-measures factors. Individual dose and day comparisons were made using post hoc Tukey honest significance tests. One-way, repeated-measures ANOVA was used to determine the statistical significance of any differences in milk-reinforced responding in the operant control group.

**Drugs.** Ketamine hydrochloride (Sigma, St. Louis, MO), xylazine hydrochloride (Fermenta, Kansas City, MO), cocaine hydrochloride (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), pentobarbital sodium (Fisher Scientific, Pittsburgh, PA), penicillin G sodium (Marsam Pharmaceuticals, Inc., Cherry Hill, NJ), and methohexital sodium (Eli Lilly and Co., Indianapolis, IN) were mixed in sterile 0.9% saline. The mAb 15A10, synthesized and purified as described previously (Mets et al., 1998), was supplied by Dr. Donald Landry and was mixed in sterile saline. All drug concentrations are expressed as the salt.

**Results.**

Over the course of testing, one rat in the 100 mg/kg antibody treatment group lost catheter patency, and data from this rat were therefore excluded from these analyses. Under these schedules and dose-access conditions, cocaine maintained different rates of responding, depending on the dose per injection. The total number of saline injections self-administered by rats was quite low in both the first and fifth (saline) components of the multiple-dose session, and these were averaged to arrive at the data in Figs. 1 through 4. Under baseline conditions (no pretreatments), there was a dose-dependent increase in injections self-administered over the three lowest doses of cocaine (0.015, 0.03, and 0.06 mg/kg/injection) and a dose-dependent decrease in self-administered injections over the three highest doses (0.125, 0.25, and 0.5 mg/kg/injection). Saline pretreatments did not influence dose-related rates of cocaine self-administration, as evi-
duced by the typical inverted U-shaped function characterizing the cocaine dose-response curve (Fig. 1), nor were the cocaine dose-response curves after saline pretreatments significantly different from those observed after baseline (i.e., no presession treatment; data not shown).

Rates of responding for cocaine self-injection varied across the cocaine dose range for each antibody pretreatment group. Cocaine dose-response curves after saline pretreatments within each antibody treatment group were not statistically different \( F(2,11) = 2.7, P = .12 \) and thus were averaged for the purpose of comparison with cocaine dose-response curves generated 30 min subsequent to 10, 30, and 100 mg/kg mAb 15A10 infusions in Fig. 1. Rates of cocaine injection varied as a function of cocaine dose for the 10 \( F(6,24) = 9.64, P < .00003 \), 30 \( F(6,24) = 2.50, P < .05 \), and 100 \( F(6,24) = 7.59, P < .0004 \) mg/kg mAb 15A10 pretreatment groups. A dose-dependent reduction in rates of cocaine self-administration was observed in the antibody-pretreated groups [main group effect: \( F(2,11) = 9.51, P < .004 \); group \( \times \) dose interaction: \( F(12,66) = 6.05, P < .000002 \)]. Whereas the acute 10 mg/kg mAb 15A10 administration did not alter the cocaine dose-response curve relative to saline, both 30 and 100 mg/kg antibody pretreatments produced statistically significant decreases in the number of injections self-administered within the 0.03, 0.06, and 0.125 mg/kg dose components of the multiple-dose schedule (all \( P < .01 \)). The 100 mg/kg mAb 15A10 pretreatment resulted in an increase in the number of self-administered cocaine infusions at the highest dose of cocaine available on the schedule (0.5 mg/kg/injection) relative to both the 10 and 30 mg/kg mAb 15A10 pretreatment groups \( (P < .004) \).

Figures 2 through 4 depict rates of cocaine-maintained responding for each mAb 15A10 pretreatment group 30 min after either saline or antibody infusion. Also shown are cocaine dose-response curves observed every day after these treatments until cocaine-reinforced responding was not statistically different from control (saline), as assessed by repeated-measures ANOVA. As antibody concentration increased from 10 to 100 mg/kg, rates of cocaine-reinforced responding, particularly over the ascending limb of the cocaine dose-response curve, were suppressed to a greater extent. The two highest doses of antibody (30 and 100 mg/kg) appeared to equally suppress cocaine-reinforced responding on the ascending limb of the cocaine dose-response curve. The 10 mg/kg antibody pretreatment (Fig. 2) was ineffective in reducing cocaine self-administration [main day effect: \( F(2,8) = 2.36, P = .16 \); main dose effect: \( F(6,24) = 9.64, P < .00003 \); day \( \times \) dose interaction: \( F(12,48) = 1.38, P = .21 \)]. The 30 mg/kg antibody pretreatment (Fig. 3) suppressed responding for cocaine on the day of treatment only [main day effect: \( F(1,8) = 3.92, P < .064 \); main dose effect: \( F(6,24) = 2.49, P < .5 \); day \( \times \) dose interaction: \( F(12,48) = 2.69, P < .01 \)]. Post hoc tests revealed a significant difference between saline and antibody pretreatment days at the 0.06 mg/kg/injection cocaine dose \( (P < .05) \). Cocaine self-injection reverted to baseline levels in the 30 mg/kg antibody pretreatment group by 24 h after infusion [main day effect: \( F(1,4) = 1.18, P = .34 \); main dose effect: \( F(6,24) = 3.6, P < .02 \); day \( \times \) dose interaction: \( F(6,24) = 0.35, P = .90 \)]. At the highest dose of 100 mg/kg, mAb 15A10 altered rates of cocaine-maintained lever pressing for at least 48 h [main day effect: \( F(4,12) = 20.99, P < .00001 \)].
P < .00003; main dose effect: $F(6,18) = 7.59, P < .0004$; day × dose interaction: $F(24,72) = 4.52, P < .000001$ (Fig. 4). Rates of responding for cocaine injection were significantly attenuated on the day of antibody pretreatment relative to saline at the 0.06 ($P < .001$) and 0.125 ($P < .02$) mg/kg cocaine unit doses. In comparison with saline pretreatment, cocaine dose-response curves observed 24 and 48 h after antibody treatment demonstrated statistically significant main dose effects as well as day × dose interactions (all $F$ tests significant at $P < .05$). However, main day effects were not statistically significant, indicating that overall rates of responding across all cocaine doses available on the multiple-dose schedule were not different from saline effects 24 and 48 h post-treatment. In the 100 mg/kg group, cocaine-maintained responding was not significantly different from baseline values by 72 h post-treatment [main day effect: $F(1,3) = 4.6, P < .12$; main dose effect: $F(6,18) = 19.15, P < .000002$; day × dose interaction: $F(6,18) = 2.56, P = .06$].

Figure 5 depicts rates of responding for the 5-s access to the sweetened milk solution in a separate group of rats ($n = 4$). Response rates were not significantly different between days in which rats were infused with 100 mg/kg mAb 15A10 and days in which rats were infused with volume-control saline [$F(1,3) = 0.77, P = .44$].

### Discussion

The results of this study replicate and extend previous work demonstrating the effectiveness of mAb 15A10 in selectively antagonizing cocaine’s effects on self-administration behavior in rats. The main finding was a dose- and time-dependent modification of the number of cocaine self-injections taken by groups treated with 30 and 100 mg/kg mAb 15A10, relative to treatment with saline and 10 mg/kg mAb 15A10. The changes in cocaine-reinforced responding were not attributable to a general behavioral disturbance, as evidenced by intact operant responding maintained by an alternate reinforcer (milk). At effective concentrations of catalytic antibody (i.e., 30 and 100 mg/kg), reductions in the number of cocaine injections taken by subjects were primarily limited to doses on the ascending limb of the cocaine dose-response curve, whereas larger doses on the descending limb were either unaffected or increased (Fig. 1). This dose-dependent effect resulted in downward and rightward shifts in the cocaine dose-response curve in the 30 and 100 mg/kg antibody treatment groups, which may be indicative of functional pharmacokinetic antagonism of cocaine by mAb 15A10. A reasonable interpretation of the decrement in rates of cocaine self-injection at lower unit doses, along with the increase in rates of self-administration at higher doses, is that mAb 15A10 administration produced a functional dose reduction across the cocaine dose-response curve. These alterations in cocaine-maintained behavior are similar to, although much greater in magnitude given the dose and session parameters than, those observed after traditional pharmacodynamic approaches, for example, dopamine antagonists (Gerber and Wise, 1989; Bergman et al., 1990; Emmett-Oglesby et al., 1993; Caine and Koob, 1994).
Mets et al. (1998) previously demonstrated a shift in the metabolic profile of cocaine in rodents after i.v. pretreatment with 100 mg/kg mAb 15A10. Using a constant catecholamine coinfusion model of cocaine toxicity, these researchers found a 3-fold increase in the LD<sub>90</sub> for i.v. cocaine in antibody-treated rats and no significant difference in plasma concentrations of cocaine at the time of death, but a 10-fold increase in the level of ecgonine methylester, the main metabolite of cocaine by the benzoyl esterolysis pathway. From these findings, it was concluded that mAb 15A10 must be exerting its protective effects through a prereceptor pharmacokinetic mechanism, specifically, through the in vivo sequestration and catalysis of cocaine in the circulation.

Although the present study is in general agreement with the findings of Mets et al. (1998), it has not replicated exactly their main findings in terms of the concentrations of antibody that were effective in decreasing cocaine self-administration and in the time course of the rate-decreasing effect. Whereas Mets et al. were able to demonstrate convincing blockade of behavior maintained by a single (0.3 mg/kg/injection) dose of cocaine with a 24-h pretreatment of mAb 15A10 in the range of 30 to 40 mg/kg, our present work revealed an effective reduction in cocaine self-administration only up to a 0.125 mg/kg/injection dose of cocaine with a 30-min antibody pretreatment.

Fig. 5. Milk-reinforced operant responding after 100 mg/kg mAb 15A10. The average (± S.E.M.) number of reinforcer deliveries (5-s access to 0.5 ml of sweetened milk) obtained on an FR5 timeout 10-s schedule of reinforcement after 30-min pretreatments of saline or mAb 15A10 are illustrated.

With respect to the differences observed in the present results compared with the results of Mets et al. (1998), some possible explanations relate to four important methodological differences between the two studies. First, the current studies implemented a multiple-dose within-session procedure, wherein subjects had access to six different unit doses of cocaine and saline within a fairly lengthy, 8-h testing session, whereas Mets et al. (1998) used a single-dose substitution methodology. Second, although the stimulus arrays and contingencies were identical within each component of our testing protocol, relative to those used in the earlier study, the duration of the training procedures and the criteria defining stability in response rates as a prerequisite to administration of the experimental pretreatments were different. Third, rates of infusion of each cocaine injection were different in that the earlier study used a relatively slow infusion pump to administer cocaine infusions, whereas we have since converted to a pneumatic syringe system that delivers a given dose injection within 1 to 2 s, compared with 5 to 6 s with an infusion pump. Fourth, and perhaps most important, the present testing protocol scheduled the automatic inclusion of priming injections of cocaine at the beginning of each dose component of the schedule equivalent to the cocaine dose the rat would normally receive in that component. The propensity of cocaine priming injections to facilitate cocaine-reinforced responding and spontaneous recovery from extinction have been well described in the literature (deWit and Stewart, 1981; Panililo et al., 1998; Norman et al., 1999; Spealman et al., 1999; Stewart, 2000). It might have been reasonably predicted that the faster infusion time of each i.v. bolus and the incorporation of priming injections that may serve as salient discriminative stimuli in the multiple-dose schedule would naturally lead to recovery of cocaine-reinforced responding at lower unit doses.

Although the present results are encouraging, several obstacles remain before the therapeutic use of mAb 15A10 or other passively administered antibodies for cocaine abuse and toxicity can be evaluated thoroughly. First, mAb 15A10 is a murine monoclonal antibody not suitable for use in humans. “Humanization” of the enzyme, that is, grafting of the complementarity determining regions into a human antibody framework, will be needed to avoid recognition by any recipient’s immune system. Second, although assessment of pharmacokinetic agents in small animals with rapid circulatory transit times may not fairly reflect performance of the agent in human adults, it is important to note that mAb 15A10 was only effective in attenuating rates of cocaine self-injection at relatively low unit doses of cocaine, up to 0.125 mg/kg/injection. Beyond this dose, mAb 15A10, at all doses tested, did not attenuate rates of cocaine self-injection. However, mAb 15A10 did slightly increase rates of responding in the 30 and 100 mg/kg antibody pretreatment groups, relative to saline pretreatment, for the two highest doses of cocaine available on the multiple-dose schedule, 0.25 and 0.5 mg/kg/injection. Such an increase may indicate that the protective effects of the antibody, with respect to cocaine reinforcement, are liable to be overcome with repetitive dosing of higher concentrations of cocaine. However, it also appears that we have not yet evaluated the dose of antibody that will afford maximal protective effects against the behavioral effects of cocaine.

It has been suggested that merely attenuating the rate of drug distribution to sites of action within the brain might be adequate to decrease the magnitude of the agent’s central effects—in the case of cocaine, attenuating its subjective and reinforcing properties (Verebey and Gold, 1988; Evans et al., 1996). It might then be predicted that simple binding antibodies might effectively reduce the physiological, behavioral, and toxic effects of cocaine by lowering brain levels of the cocaine.
drug. Indeed, this possibility has been borne out in recent work from several laboratories (Bagasra et al., 1992; Carrera et al., 1995, 2000; Ettinger et al., 1997; Fox, 1997). A catalytic antibody, even one with relatively low turnover [with reference to enzymes such as butyrylcholinesterase or to recently synthesized butyrylcholinesterase mutants such as those from Xie et al. (1999)], might be expected to have advantages over a simple binding antibody in that it would not theoretically need to be administered in roughly stoichiometric quantities to cocaine and would probably be more difficult to overwhelm with repetitive cocaine administration (Yang et al., 1996).

At the present time, anticocaine antibodies are far from optimized with respect to kinetic parameters. Noncatalytic antibodies can be viewed as a catalyst at one extreme [i.e., a catalyst with high affinity ($K_{\text{cat}}$) and infinitely low turnover ($k_{\text{cat}}$)]. If a noncatalytic antibody with a binding affinity in the range of an achievable $K_m$ for a catalytic antibody (~1 $\mu$M) were an effective pharmacokinetic blocker, then a mAb 15A10 mutant with a $k_{\text{cat}}$ no better than that of native mAb 15A10 but with a much lower $K_m$ could be an exceptionally effective agent. In fact, a recent report indicates that active as well as passive immunization of noncatalytic antibodies with binding affinity in the range of 1 $\mu$M (0.24 $\mu$M) can be very effective in animal models of cocaine self-administration. Carrera et al. (2000) have recently reported significant antagonism of cocaine self-administration (0.25 mg/kg/injection) with pretreatments of 30 to 40 mg/kg of a monoclonal antibody, GNC92H2. These researchers also reported similar antagonism of cocaine self-administration using active immunization techniques. Immunization with GNC-keyhole limpet hemocyanin significantly shifted the cocaine dose-response curve (0.015–0.5 mg/kg/injection) to the right, blocking administration of cocaine unit doses up to 0.06 mg/kg/injection while producing increases in rates of self-injection at higher unit doses (0.25 and 0.5 mg/kg/injection) of cocaine. The shift to the right in the dose-effect function that these authors found after active immunization is similar to that observed in the present study using passive administration techniques; 30 mg/kg mAb 15A10 produced a remarkably similar, approximately 8-fold, shift in the dose of cocaine required to maintain responding in antibody-treated rats. Passive immunization with GNC92H2 in the range of 30 to 40 mg/kg i.v. likewise produced significant reductions of cocaine self-administration.

Dose-response animal models of cocaine self-administration used in this and previous studies (Carrera et al., 2000), which afford relatively unlimited “binge” access to cocaine, appear to indicate a saturation point for pharmacokinetic interventions, after which additional increases in cocaine unit doses lead to a breakdown in protection from the reinforcing effects of the drug. This problem of surmountable antagonism, as noted previously, is reminiscent of findings with dopaminergic antagonists. A single dose of i.v. cocaine administered by a typical human user (25–50 mg) exceeds 0.5 mg/kg/injection (0.36–0.71 mg/kg/injection), assuming an average body weight of 70 kg (Verehey and Gold, 1988; Gatley et al., 1998). Although longer circulatory transit times in human recipients may decrease mAb binding and catalytic rate requirements, a significant improvement in mAb 15A10 kinetics is the subject of ongoing investigations to effectively antagonize the subjective and reinforcing effects of cocaine in this dose range. In light of our previous work and in view of the findings of Carrera et al. (2000), recent mutagenesis studies are focusing on producing mAb 15A10 variants with greater affinity for cocaine (i.e., lower $K_m$ rate). Such an improvement is especially important to effectively reduce the systemic and behavioral effects of cocaine doses in this range, especially given the characteristic binge pattern of repeated cocaine administration demonstrated by most users (Denison et al., 1998; Walsh, 1998). With respect to the characterization of anticocaine agents such as mAb 15A10, additional studies should also address quantitative dose-response assessment of the effects of antibody administration on in vivo cocaine concentrations, particularly within the brain, to confirm that the artificial enzyme effects are indeed mediated through prereceptor catabolism of cocaine. Comparisons of simple binding antibodies and catalytic antibodies in a variety of quantitative physiological and behavioral assays similar to those in the present methodology would also yield useful information about the impact of $K_m$ and $k_{\text{cat}}$ characteristics on antagonism of cocaine's systemic and behavioral effects.

References


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