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The effects of a repeated dose of a recombinant humanized anti-cocaine monoclonal antibody on cocaine self-administration in rats

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ABSTRACT

Background: Immunotherapy has shown potential as a treatment for cocaine abuse. The humanized recombinant anti-cocaine monoclonal antibody (mAb) with the preclinical designation h2E2 has been shown to decrease cocaine concentrations in the brain in rats, but its effects on cocaine self-administration behavior have never been tested.

Methods: The amount of cocaine needed to reinstate self-administration behavior (priming threshold) was calculated and the inter-injection intervals at unit doses of 0.3 μmol/kg and 3 μmol/kg during maintained self-administration were measured over a five-week baseline period. Rats trained to self-administer cocaine were infused with two doses of h2E2 (120 mg/kg i.v.) 35 days apart. Priming threshold and inter-injection intervals were measured for 35 days after both injections.

Results: After both injections of h2E2, priming thresholds were significantly increased (3-fold) compared to expected baseline and then gradually declined over 35 days. A significant decrease (15–33%) in inter-injection intervals during maintained self-administration was also observed following both h2E2 infusions at the lower dose, and after the first injection at the higher dose. No significant decreases in body weight were observed after either injection, indicating a lack of toxicity following a second injection.

Conclusions: These data predict that the safety and effectiveness of h2E2 will be maintained after multiple treatments of this potential immunotherapy for cocaine abuse.

1. Introduction

Cocaine reliably induces the reinstatement of self-administration behavior in rats and has been suggested to partially model relapse in cocaine addicts (Stewart, 1983; Norman et al., 1999). The minimal amount of cocaine needed to reinstate self-administration behavior was termed the priming threshold (Norman et al., 1999, 2002). Therefore, the cocaine concentration at the site of action is a critical determinant of reinstatement of self-administration behavior (Norman et al., 1999). An increase in the cocaine priming threshold is predicted to decrease the probability of a cocaine-induced relapse. One method for decreasing the amount of cocaine at its site of action (dopamine transporters in the brain) is the use of anti-cocaine antibodies, which bind to cocaine and prevent its distribution into the brain. This bound cocaine is assumed to be pharmacodynamically inert. Cocaine vaccines that induce the production of anti-cocaine antibodies have been shown to decrease cocaine use in clinical trials, but only in patients that produce high antibody titers. The responses to these vaccines are highly variable, and since their effectiveness is directly related to antibody titers, the clinical response to these medications is not uniform (Martell et al., 2009; Kosten et al., 2014). Similarly, an anti-nicotine vaccine has shown some effectiveness in clinical trials (Keyler et al., 2005), but only if high anti-nicotine titers are elicited.

An alternative to active immunization using vaccines is the use of passive immunization with anti-cocaine antibodies. Since the main predictor of success of cocaine vaccines is the amount of antibody raised, injection of known doses of a high-affinity anti-cocaine monoclonal antibody (mAb) should provide a more consistent and reliable therapeutic effect. An anti-methamphetamine mAb has shown effectiveness in animal models of methamphetamine abuse (Laurenzana et al., 2003) and has advanced into clinical trials (Stevens et al., 2014). A chimeric anti-cocaine mAb 2E2 has

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been effective in decreasing cocaine concentrations in the brain in mice (Norman et al., 2007) and has raised the cocaine priming threshold in rats (Norman et al., 2009). This was interpreted as a decrease in the probability of a dose of cocaine reinstating self-administration behavior. The recombinant humanized anti-cocaine mAb 2E2 was reengineered, and the humanized h2E2 is now a lead candidate for development as a passive immunotherapy for cocaine abuse. It shares greater than 95% sequence homology with the human IgG_1, has high affinity (K_d = 4.1–16 nM) and selectivity for cocaine, and can now be produced in gram quantities using a stably transfected mammalian cell line (Norman et al., 2014; Kirley and Norman, 2015). Although a single infusion of this mAb has been shown to prevent cocaine entry into the brain in rats (Norman et al., 2014), the effects of h2E2 on cocaine self-administration behavior have not previously been investigated. Additionally, long-term treatments for relapse prevention will require multiple doses, but the effects of a repeat injection of h2E2 nor 2E2 have been studied. Therefore, the aim of this study was to determine the effects of h2E2 on cocaine induced reinstatement of self-administration and maintained self-administration in rats and to investigate whether a repeat dose remains effective.

2. Materials and methods

2.1. Animals

17 male Sprague-Dawley rats between 200 g and 500 g during the course of this study were purchased from Harlan Laboratories (Indianapolis, IN). Rats were housed individually on a 14/10-h light/dark cycle with unrestricted access to food and water. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011) and under a protocol approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

2.2. Self-administration training

Rats were implanted with indwelling catheters into the right jugular vein under isoflurane anesthesia. If recatheterization was required, catheters were placed in the left jugular and femoral veins as needed throughout the study. Buprenorphine (0.03 mg/rat s.c) was administered post-surgery for pain control and gentamycin (25 mg/rat s.c) for three days was used to prevent infection following surgery. Detailed protocols for cocaine self-administration training can be found in Tsibulsky and Norman (2005). In brief, beginning at least 5 days after surgery, rats were trained to self-administer cocaine HCl. Rats were weighed immediately prior to each self-administration session. Animals were placed in isolated chambers containing an active and an inactive lever. During training, a unit dose of 3 μmol/kg (approximately 1 mg/kg) of cocaine HCl was delivered on a fixed-ratio 1 (FR1) schedule with at least a 5 s timeout period. A cue light was illuminated for the duration of the timeout. Rats had access to cocaine for 3 h a day, five days a week. Training was considered complete when inter-injection intervals did not systematically deviate (defined as less than ±10% variance (standard deviation) in the mean inter-injection interval of a standard unit dose of cocaine (3 μmol/kg)) from day to day for three consecutive sessions.

2.3. Priming threshold

Sessions began between 8:00 and 10:00 A.M., 6 days a week. Priming threshold, defined as the minimal level of cocaine that reinstates self-administration behavior, was estimated using programmed escalating doses of cocaine to raise the concentration in the rat until self-administration was reinstated. A procedure modified from Norman et al. (1999, 2009) was used. In our previous studies, the concentration of cocaine during the priming phase of the session was increased linearly. In this study, the concentration was increased according to a sigmoid logistic function with the maximum level set at 20 μmol/kg to prevent overdose if the rat never met the criterion for reinstatement.

First, rats were placed in the chamber and a cue light associated with cocaine injection was illuminated after every active lever press and at variable intervals of 100–600 s until no lever presses occurred for at least 30 min. This was done to eliminate the interference of cue-induced lever pressing with the measurement of priming threshold. Once cue-induced lever-pressing was extinguished, programmed non-contingent injections of cocaine were given every two minutes at escalating doses in order to raise the concentration of cocaine. When the rat pressed the active lever 5 times with each interval shorter than 2 min, it was defined that self-administration had been reinstated and programmed injections ceased. Priming threshold was determined by averaging the calculated peak cocaine levels after the second to last and last priming injections.

2.4. Maintained self-administration and extinction

After self-administration was reinstated, the program allowed the rat 75 injections of 0.3 μmol/kg and then 15 injections of 3 μmol/kg. When these injections were complete, the syringe pump was inactivated and lever pressing was recorded but did not result in an injection. Animals were left in their chambers until 30 min had passed since their last lever press, at which time the session was ended. Animals were then removed from the chamber and returned to their home cages until the next session.

2.5. Calculations of cocaine concentration in the body

Complete protocols for the calculation of cocaine in the rats' bodies can be found in Tsibulsky and Norman (2005). Briefly, the drug level in the body was calculated every second using a one-compartment pharmacokinetic model with an assumption of a 500 s elimination half-life of cocaine.

2.6. Quantification of cocaine and benzoylecgonine following maintained self-administration

A different set of rats trained to self-administer cocaine was given access to cocaine (fixed ratio FR1) with a unit dose of 3 μmol/kg for 4–5 h. At the end of a session, a small incision was made at the tip of the tail and 10–50 μL of blood was collected. Cocaine and BE were extracted using solid phase extraction and quantified using GC/MS as previously reported in Norman et al. (2007).

2.7. h2E2 infusions

The mAb h2E2, concentrated to 17 mg/mL in phosphate buffered saline (pH 7), was infused using the same apparatus used for the self-administration studies. Antibody (120 mg/kg, i.v.) was infused at a rate of 162 μL/min, where the duration of infusion was adjusted for the rats' body weight. Infusions lasted approximately 20 min.

2.8. Data analysis and statistics

Due to multiple catheter failures, six out of twelve rats were eliminated from the experiment before the first h2E2 injection and one rat was eliminated before the second injection of antibody. Every daily session generated four measurements: the body weight,