Titer-Dependent Antagonism of Cocaine following Active Immunization in Rhesus Monkeys

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ABSTRACT

Immunization may be a useful pharmacokinetic antagonist therapy for cocaine users. Three rhesus monkeys were immunized with a cocaine:bovine serum albumin conjugate in alum and later with complete and incomplete Freund’s adjuvants. Monkeys developed cocaine-binding antibodies (as measured by enzyme-linked immunosorbent assay) after immunization with alum; greater antibody titers developed after immunization with Freund’s adjuvants. The response rate-decreasing effect of cocaine diminished in proportion to antibody titer; there was no substantial change in the rate-decreasing effect of bupropion. Plasma cocaine concentrations increased in proportion to antibody titer. Immunizations were well tolerated and had no effect on response rates. These data suggest that the antibody response to a cocaine antigen can produce a specific pharmacokinetic shift in cocaine distribution sufficient to antagonize a behavioral effect of the drug, and can do so with minimal side effects.

Cocaine abuse is a medical and social problem (Johanson and Schuster, 1995). Although many users are motivated to quit, a substantial number are not successful; adjunctive pharmacotherapy, particularly antagonist drugs, would be useful for these individuals. However, the multiple protein targets of cocaine action and the inhibitory mechanism of action on these proteins make traditional receptor antagonist development unlikely (cf. Billman, 1990). Under these circumstances, pharmacokinetic antagonists, interventions that modify the distribution or metabolism of cocaine, may be more likely to succeed. A strategy directed at the drug molecule, such as immunization, avoids the question of the drug’s mechanism of action.

Immunological antagonism of cocaine has been accomplished in rodents. Using keyhole limpet hemocyanin as a carrier protein and Freund’s adjuvant, it was found that rats produced antibodies against cocaine and showed less analgesic effect from cocaine (Bagasra et al., 1992). However, antagonism was neither complete nor well predicted by antibody titer. In another attempt, the same adjuvant and carrier protein but a different linker between cocaine and the carrier was used. These rats developed antibodies and showed less analgesic effect of cocaine (Ettinger et al., 1997); however, conditioned place preference did not show clear antagonism.

A third group immunized rats with the same carrier protein but with an experimental adjuvant designed to be less toxic than Freund’s. They reported antibody formation and reduced cocaine-induced locomotor activity as well as reduced cocaine content in brain (although neither activity nor drug was eliminated; Carrera et al., 1995). Finally, a fourth group immunized mice with Freund’s adjuvants but used bovine serum albumin as a carrier. Their mice developed antibodies against cocaine; when these antibodies were purified and given to rats trained to self-administer cocaine, the rats responded as if the cocaine dose had been reduced (Fox et al., 1996).

Although encouraging, these studies leave several gaps in our knowledge. First, if the antibody response mediates the antagonist effect of immunization, then the strength of the response should predict the degree of antagonism. This usually has not been measured and has not been demonstrated clearly. Second, all of the studies reported to date rely on immunizations in fairly homogenous populations of rodents. Experiments have not been attempted in heterogeneous populations (e.g., mixed sex, outbred, different ages) or in species from other orders (e.g., primates). Although demonstration of an immunization effect in any setting would be interesting, a positive finding in a more challenging subject population would be more interesting. As an outbred species with different immunological histories, rhesus monkeys can be expected to use a diverse array of genes to form an antibody response.

ABBREVIATIONS: SRV, simian retrovirus; ELISA, enzyme-linked immunosorbent assay; CD, cluster of differentiation protein; BSA, bovine serum albumin; CFA, complete Freund’s adjuvant; IFA, incomplete Freund’s adjuvant; HPLC, high pressure liquid chromatography.
Clear antagonism in such a group would be more interesting than a comparable finding in other species. Third, the role of the adjuvant in production of cocaine-binding antibodies has only been briefly addressed by one group. These authors indicated that there was no difference between alum and Freund's adjuvants (Fox et al., 1996). However, the literature strongly suggests that alum is a less effective adjuvant (Sacco et al., 1989; for review, see Siegrist and Lambert, 1999) with a side effect profile including a lesser tendency to produce lesions but a greater tendency to produce reaginic antibodies and hypersensitivity reactions (Alving et al., 1996).

The experiments described below sought to address these issues by immunizing three outbred rhesus monkeys of different age and sex with a cocaine conjugate. Immunizations began with an alum adjuvant; after a hiatus, immunizations were conducted with complete and incomplete Freund's adjuvants. Blood samples were taken and antibody titers measured for comparison with the operant behavior suppressant effect of cocaine and bupropion. Suppression of food-maintained responding was chosen because the lab has found it to be an efficient measure of drug effects (Winger et al., 1994).

A small number of cocaine administrations was conducted outside of operant sessions for determination of plasma cocaine concentrations. Subjects were also monitored for development of side effects from immunization.

**Materials and Methods**

**Subjects.** Subjects were three rhesus monkeys (Macaca mulatta; two male, one female) maintained in a temperature- and humidity-controlled colony room. All subjects had previously been exposed to cocaine and other drugs. At the beginning of the experiment, one male weighed 9.0 kg; the other monkeys weighed 5.0 kg. Water was available ad libitum in the home cages; after operant sessions, monkeys were given the maximum amount of chow that would not interfere with control response rates, and supplemented with fresh fruit. Two of the monkeys were in apparent good health and tested negative for serum antibodies to antigens from simian retrovirus (SRV) types 1 to 5 and macaque, African green monkey, and sooty mangabey strains of simian immunodeficiency virus (testing by enzyme-linked immunosorbent assay (ELISA), conducted by Virus Reference Laboratory, San Antonio, TX). The third monkey tested positive for antibodies to SRV-2. Further testing was conducted to compare with published descriptions of SRV pathology in macaques, which describe a suppression of both CD4-positive and CD8-positive T lymphocytes and immune compromise (Gardner et al., 1994). A differential white blood cell count was performed at the beginning of the experiment by the University of Michigan Unit for Laboratory Animal Medicine Diagnostic Lab. Although the total white blood cell count was within normal limits at 7.0 × 10^9/l, the lymphocyte percentage was significantly below normal (30%; reference mean ± S.D., 51.5 ± 7.3). A flow cytometry analysis of lymphocytes using antibodies to CD8 (T-cell receptor-associated protein, a marker for T cells), CD4 (major histocompatibility complex II coreceptor, a marker for helper and inflammatory T cells), and CD8 (major histocompatibility complex I coreceptor, a marker for cytotoxic T cells) was performed by the New England Regional Primate Research Center (NERPRC) Division of Immunology (Southborough, MA). The results showed an approximately normal ratio of CD4-positive to CD8-positive cells (2173:1193) among cells expressing CD3. This suggests suppression of both CD4-positive and CD8-positive T cells. In addition, this monkey developed two separate cases of gastroenteritis, which did not spread to monkeys not infected with SRV or simian immunodeficiency virus. These findings are consistent with active SRV-2 infection (Gardner et al., 1994).

**Behavioral Protocol.** Experimental sessions were carried out 5 days/week. Monkeys were placed in Plexiglas and tubular aluminum primate restraint chairs for handling and experimental sessions. The chairs were placed in chambers equipped with one lever on each side of a food cup. A pellet dispenser delivered 300 mg of banana-flavored pellets (P.J. Noyes, Lancaster, NH) to the cup as reinforcers. The chambers were equipped with light display panels located above the levers. Ventilation and background masking noise was provided by an exhaust fan that ran continuously. Responses were recorded and contingencies were programmed with an IBM pC microcomputer (Armonk, NY). Monkeys were trained to press levers by the method of successive approximations. Response requirements were increased through progressively higher fixed ratio values. After responding stabilized on a fixed ratio-20 schedule, contingencies alternated on a cycle of time out and food availability. Gradually, the number of cycles per session was increased (to 10 cycles) as the number of pellets delivered and the amount of time per cycle were decreased. Each cycle began with a 5-min time-out period when no stimulus lights were illuminated. All injections were given intramuscularly in the thigh during the 1st min of the time-out period. Responses during the time-out period were not recorded or reinforced. The time-out period was followed by a 5-min response period in which two green lights were illuminated. After 30 responses, a reward pellet was delivered; responses on either lever were counted. The response period ended either when the subject received 10 pellets or the maximum time of 5 min was reached. Control rates of responding were determined from sessions in which an injection of saline was administered before the first cycle. Control rates are the average of the response rates for all 10 cycles. Although control rates varied from 1.7 to 4.8 responses/s across subjects, these rates were consistent (within ±0.75 responses/s) for each individual. Drug trials were scheduled if the control rate was greater than 1 response/s on the day before the drug trial. In drug trials, an injection of the vehicle for the drug was administered during the cycle before the first drug injection. Drugs were not administered if the response rate following vehicle administration was less than 1 response/s. Injections were intramuscular and contained either cocaine hydrochloride (National Institute on Drug Abuse Research Technology Branch, Rockville, MD) or bupropion hydrochloride (Glaxo Wellcome, Research Triangle Park, NC); doses are presented as the salt forms. The lowest dose of each drug that reliably suppressed responding was chosen for use in the study.

**Immunizations.** The antigen used for these studies was a BSA: cocaine conjugate (molar ratio 1:7). The tropine nitrogen of norcocaine was activated with succinic anhydride; the resulting carboxylic acid was coupled to BSA. The antigen was diluted in sterile saline to 1 mg/ml and mixed 1:1 with the adjuvant. The first course of immunizations used alum (Pierce, Rockford, IL) as an adjuvant. The antigen was adsorbed to the alum by continuous agitation for 20 to 30 min; the mixture was shaken to ensure an even suspension before injection. The second course of immunizations used complete Freund's adjuvant (CFA; Sigma, St. Louis, MO) for the first immunization and incomplete Freund's adjuvant (IFA; Sigma) for subsequent immunizations. These adjuvants were mixed with the antigens using two glass syringes and a mixing needle (Popper, New York, NY). For all immunizations, 200 μl of the mixture (containing 100 μg of antigen) was injected subcutaneously at a shaved site on the monkey's back. Immunizations were made at 4-week intervals. Drug tests were conducted 2 and 4 weeks after immunization (i.e., halfway between immunizations and immediately before the next immunization). Blood was collected from the saphenous vein at weekly intervals, immediately after an operant session. Samples were collected into 2-ml tubes containing EDTA and separated by centrifugation; plasma aliquots were stored at −80°C until analysis. Skin tests were performed after operant sessions 2 weeks after immunizations; the protocol was derived from Norman and Peebles
(1997). Approximately 50 μl of a solution containing saline, cocaine, or histamine (Sigma) was injected intradermally in a shaved area on the arm. At various intervals, the area of any wheal formed was measured by taking perpendicular measurements with a ruler. Skin tests were performed during the alum immunization series due to the tendency of alum-containing vaccines to produce IgE titers out of proportion to their IgG response. The same subjects were immunized with the antigen in both adjuvants; immunizations with alum spanned 6 months, whereas the Freund’s series required about 3 months. A period of 6 months passed between alum and Freund’s immunizations; a period of 5 months passed between Freund’s immunizations and follow-up experiments.

**Immunoassays.** Antibody titers were measured by ELISA. A cocaine-ovalbumin conjugate, prepared in the same manner as the BSA conjugate, was coated on microtiter plates (Corning, Corning, NY) overnight at 4°C at a concentration of 20 μg/ml. Plates were blocked with 3% ovalbumin (Sigma) for 2 h at room temperature; before and after blocking, plates were washed twice with chilled phosphate-buffered saline. Plasma samples were diluted in 3% ovalbumin and incubated for 2 h at room temperature. Secondary antibodies diluted in 3% ovalbumin were also incubated for 2 h at room temperature; before and after secondary incubation, plates were washed three times with chilled phosphate-buffered saline. The solution used for routine detection was an alkaline phosphatase-coupled antibody against macaque IgG (Sigma) diluted 1:10,000. The alternate solution was an alkaline phosphatase-coupled antibody against mouse IgG (Southern Biotechnology Associates, Birmingham, AL) diluted 1:10,000. After plates were dried, activity was measured with para-nitrophenol phosphate (Sigma), incubated for 25 min at room temperature; results were read as absorbance at 405 nm using a microplate reader (Molecular Devices, Menlo Park, CA).

**HPLC.** The protocol used in the current study was adapted from published methods (Jatlow and Nadim, 1990). The HPLC system used for determination of plasma cocaine levels was comprised of a solvent delivery system (Waters 600E, Milford, MA), UV detector (Waters 486), and injector fitted with a 20-μl injection loop (Waters 717+). The HPLC system was fitted with a 3.9-×150-mm Waters Symmetry C8 column (5-μm particle size), and was controlled with Waters Millenium software. Chemicals were purchased in HPLC grade from Fisher Scientific (Springfield, NJ) except as noted. The mobile phase consisted of a solution of 333 ml of acetoniitrile and 1 liter of 50 mM phosphate buffer (6.9 g of KH₂PO₄ in 1 liter of distilled water, adjusted to a pH of 3.0 with ortho-phosphoric acid) containing 1.88 g/l sodium hexanesulfonate as an ion-pairing reagent. The mobile phase was vacuum filtered (47-mm nylon, 0.22-μm filter membrane; Micron Separations, Westboro, MA) and degassed under vacuum for 20 min. A carbonate buffer (700 mg of a dry mixture of 20 g of Na₂CO₃ and 17.5 g of NaHCO₃, constituted in 10 ml of deionized water) was used to extract samples. Chromatography was performed with a mobile phase flow rate of 1.0 ml/min, and the effluent was monitored at 235 nm with sensitivity and rise time set at 0.01 absorbance units full scale and 1.0 s, respectively. Whole blood samples to be analyzed for cocaine content were collected into ice-cooled 2-ml Vacutainer tubes containing ethylenediaminetetraacetic acid and 100 μl of a saturated sodium fluoride solution. Samples were centrifuged at 3100 relative centrifugal force for 10 min, after which 1 ml of plasma was transferred to a cryostorage vial, frozen, and stored at −80°C. Two 400-μl aliquots of plasma were extracted for measurement of cocaine. These samples and standards were prepared for extraction by the addition of 50 μl of lidoacaine as an internal standard (Sigma; 10,000 ng/ml in methanol), 50 μl of carbonate buffer, and 1.2 ml of a isomyl alcohol:hexane (20 ml:1.0 liter) solution. Samples were then agitated for 15 min and centrifuged at 3100 relative centrifugal force for 7.5 min. The upper (organic) layer was transferred to a new tube containing 40 μl of 0.1 N HCl, agitated for 5 min, and centrifuged for 5 min. The organic layer was discarded, and the lower (aqueous) phase injected into the HPLC. Standard cocaine solutions (1000-, 500-, 250-, 100-, 50-, and 25-ng/ml concentrations) were prepared from a stock solution of 1 mg/ml cocaine (calculated as the salt) mixed in deionized water, and extracted in the same manner as the unknown samples.

**Data Analysis.** Due to the interindividual variation in response rates, all rate data were normalized to the data from the previous day and expressed as a percentage of control. The significance of differences in response rate was established by using paired t tests on the data collected at 10-min intervals. The effect of 0.3 mg/kg cocaine on behavior before immunization was compared with the effect of the preceding saline injection. Rates 5 and 15 min after injection were significantly different from rates after saline; the rate 25 min after cocaine was not significantly suppressed. Since the preimmune cocaine effect was limited to the 5- and 15-min observations, to limit the number of statistical tests, only data from time points up to 15 min after injection were analyzed further (e.g., for preimmune versus immune comparisons). For regression analyses, the 5- and 15-min data were averaged to produce one measure of response rate. Observations of response rate and antibody titer were organized in order of titer and collapsed into six points. Antibody titer was averaged to create an x-value, and response rate was averaged to create a y-value. The data were then analyzed by nonlinear regression using the following equation:

\[
\text{rate} = \frac{\text{spread}}{1 + \left(\frac{150}{\text{titer}}\right)}
\]

where rate and titer are the dependent and independent variables, spread is the range of response rates predicted, t50 is the titer at which a half-maximal change in response rate is predicted, and slope is a slope parameter. Goodness of fit was evaluated by calculating R² values. All statistics and regressions were calculated using Statistica 4 for Windows (StatSoft, Oklahoma City, OK).

**Results**

Plasma samples drawn at weekly intervals were analyzed by ELISA for cocaine-binding IgG. Preimmune samples and controls generally yielded absorbance values of 0.08 or less; the maximum signal observed under the conditions used for these assays was between 0.9 and 1.0. The ELISA signal could be eliminated by omission of the plasma sample, the secondary antibody, or the p-nitrophenylphosphate indicator solution (data not shown). The signal was almost eliminated by preincubation of the secondary antibody with 20-μg/ml reagent grade macaque gamma globulin (data not shown). The signal was not unique to the Sigma antibody, because a different secondary antibody from Southern Biotechnology Associates reproduced the signal (data not shown). The controls are all consistent with absorbance in this assay representing bound plasma IgG. Although plasma samples contained antibodies to BSA, titers of antibodies to ovalbumin were lower and were eliminated by the presence of 3% ovalbumin in the diluent (data not shown). Therefore, the antibody signal appears to represent cocaine-binding IgG.

All three animals responded to immunization with the antigen in alum by developing cocaine-binding antibodies (Fig. 1). The monkey with SRV-2-induced immunodeficiency is monkey 1; antibody titers for this animal are directly comparable to titers from the other two animals. One animal, monkey 3, responded to the first injection with a rapid and profound development of antibodies; this individual gradually developed titers consistent with the other monkeys. The other two subjects showed a slight and gradual increase in antibody titer from the first immunization to a plateau at the
third boost. From 4 to 13 weeks after the last boost with alum, the average titer for the three monkeys decayed in an exponential manner \((R^2 = 0.877)\) with a \(t_{1/2}\) of 1.6 weeks.

No reactivity to cocaine could be demonstrated in skin testing. Throughout the course of immunization with the antigen in alum adjuvant, intradermal injection of cocaine (5 \(\mu\)g in 50 \(\mu\)l) resulted in a wheal comparable to that remaining after saline injection (Fig. 2). In contrast, far smaller doses of histamine (0.01 \(\mu\)g at the beginning of the immunizations, 0.1 \(\mu\)g at the end) produced much larger wheals. Observation of the monkeys revealed vigorous scratching at the injection site after histamine injection but not following cocaine or saline injections. No wheal was noted at 24 h after any injection.

At 6 months, titer had returned to baseline in two of the three monkeys and nearly to baseline in the third (monkey 1; Fig. 3). After a 6-month interval, these animals were immunized with the antigen in CFA and boosted twice with the antigen in IFA. Although these observations are based on plasma diluted 1:1000, a similar pattern of results (monkey 1 above baseline but below the highest observed titer, monkeys 2 and 3 near baseline) was seen with plasma diluted 1:100. The immunization with CFA raised titers to an intermediate level, whereas the subsequent immunizations with IFA raised titers to a plateau. Neither the SRV-2 immunocompromised subject (monkey 1) nor the others showed a unique pattern of titers. The data shown in Fig. 2 are for assays at a plasma dilution of 1:1000; at lower dilutions, absorbance values were at the end of the linear range of assay results. Since the samples taken during alum immunization yielded absorbance values within the linear range at a dilution of 1:100, the immunizations with Freund’s adjuvant resulted in higher antibody titers.

Immunizations using both alum and Freund’s adjuvants were generally well tolerated with few side effects. Two of the monkeys (the 9-kg male and the 5-kg female) maintained their body weights throughout both courses of immunizations; the third monkey (the 5-kg young male) gained weight over the course of the study (data not shown). Response rates were not affected by either adjuvant either during the session immediately after immunization (Fig. 4A) or over 4 days following immunization (Fig. 4B). The mean rate of responding for the three monkeys was stable throughout the course of the experiment (Fig. 4C). The only side effect or clinical sign noted was the appearance of masses at the immunization sites. After the third or fourth booster immunization with the alum mixture, this took the form of small raised masses (1–2 cm² in area) with no signs of alopecia or irritation. In contrast, raised masses were noted after each immu-
These masses were red but the monkeys were never observed scratching them. Monkeys did not react to an experimenter touching either type of mass.

Before immunizations began, injection of 0.3 mg/kg cocaine completely suppressed operant responding 5 min after injection (two observations per monkey; Fig. 5A). Response rates recovered to 24% of control 15 min after injection and to 75% 25 min after injection. Rates after saline were significantly different from the 5-min data \( t(5) = 6.43, p < 0.005 \) and the 15-min data \( t(5) = 4.10, p < 0.01 \) but not the 25-min data \( t(5) = 0.77, p = 0.47 \). Two observations from each monkey at the time the highest antibody titers were observed were compared with the preimmune data. In the trials corresponding to peak antibody titers, the difference between rates after saline and 5 min after cocaine (54% of control) were no longer statistically significant \( t(5) = 1.39, p = 0.22 \). Although the difference between high titer and preimmune data was not significant 5 min after injection \( t(5) = 1.96, p = 0.10 \), the difference was significant at 15 min after injection \( t(5) = 3.32, p < 0.05 \). There was no evidence of a prolonged effect of cocaine after immunization. On follow-up (5 months after the end of Freund’s immunizations), titers had decreased to different degrees in all three monkeys; the effect of cocaine was variable but had partially recovered from peak immune levels (data not shown). Injection of 3.2 mg/kg bupropion suppressed behavior before and after immunization (Fig. 5B); there was no systematic change in responding as a function of immunization.

The results of each cocaine trial were reduced by averaging the response rates 5 and 15 min after cocaine. The data were sorted by antibody titer and grouped into six points; response...
rates and ELISA absorbance values at 1:1000 were averaged (Fig. 6). Nonlinear regression yielded estimates of 103% for the spread between maximum inhibition and minimum inhibition, approximately equal to the theoretically possible range; the sigmoidal model fit well ($R^2 = 0.956$). Half-maximal antagonism was predicted at an ELISA titer of 0.16 at 1:1000.

Venous plasma cocaine concentrations 15 min after injection of 0.3 mg/kg cocaine (intramuscular) increased with immunization (Fig. 7A). Because immunization with alum produced lower antibody titers and lower plasma cocaine concentrations than immunization with Freund’s adjuvant, the correlation of titer and plasma cocaine was measured (Fig. 7B). The relationship reached statistical significance [$R^2 = 0.593$; $t(11) = 3.81, p < 0.005$]. For the samples tested, the relationship between plasma cocaine and the response rate following cocaine administered on the previous day did not reach statistical significance [$R^2 = 0.236; t(11) = 1.76, p = 0.11$; data not shown].

**Discussion**

These experiments have confirmed the findings of other investigators (Bagasra et al., 1992; Carrera et al., 1995; Fox et al., 1996; Ettinger et al., 1997) and extended them in several important directions. Immunization with a hapten: protein conjugate resulted in the development of antibodies recognizing cocaine and also resulted in a decrease in a behavioral effect of cocaine (Figs. 1, 3, and 5A). Fox et al. (1996) showed in vitro specificity for cocaine; antibody binding in an ELISA assay showed less affinity for the cocaine metabolites benzoylcegonine and ecgonine methyl ester and no affinity for the structurally different local anesthetics lidocaine and procaine. However, specificity was not measured in vivo. We demonstrate specificity for cocaine relative to an equiactive dose of the structurally distinct catecholamine uptake inhibitor bupropion (Fig. 5B). Fox et al. (1996) also showed a decrease in brain cocaine concentrations; these latter data are consistent with the findings of decreased brain cocaine concentrations by Janda and colleagues (Carrera et al., 1995). Although we did not measure cocaine in the monkey’s brain, we observed increases in plasma cocaine concentrations consistent with a redistribution of the drug from tissue to circulating antibody (Fig. 7).

We have demonstrated a clear link between antibody titer and antagonist effect (Fig. 6). Previously, only one group investigated the link between antibody response and antag-

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**Fig. 6.** Decrease in the effect of cocaine was proportional to the antibody response observed. Response rate represents an average of the rates 5 and 15 min after injection. Antibody titer represents the antibody signal for a plasma dilution of 1:1000. The observations for all three monkeys were pooled ($N = 61$) and sorted into six groups according to antibody titer; observations within groups were then averaged. The resulting means for both titer and rate measurements are plotted with standard errors. Inset, individual points averaged for use in the regression are plotted with symbols representing the same subjects as in Figs. 1 and 3.

**Fig. 7.** A, cocaine concentrations observed in venous plasma 15 min following injection of cocaine (0.3 mg/kg i.m.) before immunizations began (one observation per monkey) were less than those observed during immunizations with alum adjuvant (two observations per monkey); these were less than those observed during immunizations with Freund’s adjuvant (one observation per monkey). B, plasma cocaine concentration was predicted by antibody titer. Observations collected before immunization are shown in gray, observations following immunizations using alum are shown open, and observations following immunizations using Freund’s adjuvants are shown filled.
antagonist effect. Bagasra et al. (1992) reported that titer predicts antagonism with 37% of the variability accounted for by a linear model. As long as the concentration of antibody binding sites in plasma is similar to the concentration of cocaine in plasma, the effect of binding site number will be linear; however, if either antibody or drug is present in excess, the relationship will reach an asymptote. A sigmoidal model should therefore provide a better fit over a broad range of antibody titers. Indeed, a linear model accounted for 91% of the variability in our data, compared with 96% for a sigmoidal model. The remaining difference between our data and Bagasra’s data can probably be explained by two procedural differences: the use of a larger number of observations from a smaller number of subjects and averaging data into clusters. Both of these are likely to have helped to clearly reveal the relationship between titer and antagonism. Indeed, as can be seen in the inset to Fig. 6, there is a substantial amount of scatter in the data. This situation would probably be improved by increasing the number of subjects. Although the error in the regression might be explained by a combination of the variability in the immunological and behavioral measures, there is a third potential source of error. In these experiments, measurements of antibody affinity were not feasible. However, it is possible that observations of high titer and a strong cocaine effect or low titer and little cocaine effect are due to very low- and very high-affinity antibodies, respectively.

We were also able to demonstrate a link between antibody titer and the amount of cocaine present in plasma (Fig. 7B). Assuming that elevated plasma concentrations reflect a pharmacokinetic shift from tissue to plasma antibodies as reported by Fox et al. (1996), the extent of this shift depends on antibody titer. We propose that antibody titer determines the degree of antagonism by determining the distribution of drug between tissue and plasma. These data also extend findings of a cocaine-binding antibody response to a primate model. Rhesus monkeys have much in common with humans, and have a long history in drug abuse research (Tatum and Seever, 1929). The monkeys in this study provide a mix of sexes (two male, one female) and levels of immune system function (two healthy, one immunocompromised). This is not the first report of hapten antibody formation in subjects with a below-normal number of helper (CD4-positive) T cells (Gruchalla et al., 1998). However, the strength of the antibody response in the face of clinical immune compromise is striking. This is important because of the incidence of human immunodeficiency virus infection among people using cocaine via intravenous use (Chaisson et al., 1989) or other routes (Sterk, 1988).

The magnitude of the antibody response depended on the adjuvant used to formulate the injection; CFA followed by IFA appeared to be far more effective than alum (Figs. 1, 3, and 7A). This is consistent with data on protein immunization in squirrel monkeys (Sacco et al., 1989) and with reviews on the subject (Alving et al., 1996; Siegrist and Lambert, 1999). However, it is not consistent with the statement by Fox et al. (1996) that alum and Freund’s adjuvants are equally effective. Although it is theoretically possible that this represents a species difference, preliminary data from mice immunized with BSA in either alum or CFA followed by IFA are also at odds with the Fox report. The explanation for this discrepancy is not clear.

We have also shown that these immunization regimens are safe, with few side effects. No systemic effects were noted; subjects maintained or increased their weights; response rates in control sessions did not vary systematically after immunization (Fig. 4); and no hypersensitivity reactions were noted in a skin test series during immunization with alum (Fig. 2), the adjuvant most likely to result in the development of reaginic antibodies (Alving et al., 1996). One concern that has been raised in the literature is that since antibody-hapten interactions are not covalent, the antibodies that bind a toxin in plasma will provide a reservoir of toxin and produce diminished but extended toxicity (Terrien et al., 1990). There was no sign of extended toxicity in these data. The only side effect noted was the development of masses at the immunization site. This has been reported for both Freund’s adjuvant (Alving et al., 1996; Siegrist and Lambert, 1999) and after repeated immunizations with alum (Garcia-Patos et al., 1993). It should be possible to avoid or minimize the formation of these masses by the use of synthetic adjuvants derived from Freund’s adjuvant (Alving et al., 1996; Siegrist and Lambert, 1999).

In summary, these data show that immunization produces an effective and specific antagonism of a behavioral effect of cocaine. These effects were demonstrated in a small but heterogeneous sample of rhesus monkeys, including one immunocompromised subject. Analysis of plasma cocaine concentrations suggests that antagonism depends on a pharmacokinetic shift of cocaine from its site of action in tissue to antibody binding sites in plasma. Titer (and antagonism) depended on the adjuvant used; the expression of the site effect also depended on the adjuvant. The antagonist effect could be demonstrated against a dose of 0.3 mg/kg cocaine with an antigen designed to elicit antibodies that bind cocaine. Since the effect was achieved with noncatalytic antibodies, it is tempting to speculate on the antagonist effect that could be achieved with catalytic antibodies. Such antibodies would inactivate several moles of cocaine per mole of antibody, instead of the maximum of 2 moles, which could be inactivated by a binding antibody. They might therefore be able to antagonize cocaine over a broader range of doses and routes of administration. With thorough evaluation of different adjuvants and antigens, immunization may be a safe and effective way to produce pharmacokinetic antagonism of cocaine.

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