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Effects of Passive Immunization Against Morphine on Heroin Self-Administration[1]

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KILLIAN, A. K., K. BONESE, R. ROTHBERG, B. H. WAINER AND C. R. SCHUSTER. *Effects of passive immunization against morphine on heroin self-administration.* PHARMAC. BIOCHEM. BEHAV. 9(3) 347–352, 1978.—Anti-morphine antibodies produced in Rhesus monkeys immunized with morphine-6-hemisuccinate-BSA were passively administered to recipient monkeys trained to self-administer heroin and cocaine. Following antibody administration, changes in heroin self-administration behavior were observed which were similar to those achieved with low doses of naloxone. Both manipulations increased heroin self-administration without affecting cocaine responding.

Naloxone Morphine antibodies Self-administration

ANTISERA raised against exogenous narcotic analgesics [19, 20, 22, 23] and endogenous opioid peptides [14,18] have provided useful tools for measuring the concentrations of such substances in biologic fluids and tissues, and for histochemical studies. Antibodies are also potentially useful as specific antagonists of biologically active molecules [4]. We have previously shown that antisera reactive with morphine will either prevent or reverse its depressant actions on the electrically-stimulated contractions of the guinea pig ileum [24]. Further, active immunization of a monkey trained to self-administer heroin and cocaine resulted in extinction of heroin self-administration without affecting that of cocaine [2]. In the latter study, the experimental animals continued to manufacture antibody throughout the course of the experiment and the circulating antibody activity was such that the dose of heroin required to reinitiate self-administration behavior was tenfold greater than the original reinforcing dose. The reinforcing efficacy of a drug can change in response to dose or the presence of specific antagonists [1, 7, 9, 26]. Additional variations may be introduced by the painful chronic inflammatory site necessitated by active immunization with Freunds adjuvant.

The present study was designed to eliminate some of the variables mentioned above by measuring the initial and temporal behavioral effects of an intravenous infusion of specific antimorphine antibodies in nonimmune animals trained to self-administer heroin and cocaine. This approach allowed correlation of the behavioral effects observed with both circulating antibody activity and its rate of clearance from the circulation.

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MATERIALS AND METHODS

Preparation and Characterization of Antibody

Antisera reactive with morphine were raised in two Rhesus monkeys by immunization with morphine-6-hemisuccinate-bovine serum albumin (M-6-HS-BSA). Preparation of the hapten-protein conjugate has been previously described [22,23]. Each animal received an initial subcutaneous injection of 10 mg of M-6-HS-BSA dissolved in 1 ml of phosphate buffered saline (PBS; 0.01 M PO_4, 0.15 M NaCl, pH 7.2) and emulsified in an equal volume of complete Freund's adjuvant. Secondary immunizations given on alternate weeks contained 5 mg of M-6-HS-BSA emulsified in incomplete Freund's adjuvant. Animals were bled on alternate weeks, the serum was separated from the whole blood, and stored at -20° until used. Antibody from these animals has been shown to bind heroin equally as well as morphine [21].

Pools of either whole antisera or normal monkey sera were passively transferred to the recipient monkeys for the behavioral studies described below. Prior to transfer, protein aggregates were removed by centrifugation at 10,000×G for 60 min followed by passage through 5 μ filters (Millipore Corp., Bedford, MA 01730). In some experiments, monkeys subsequently received the globulin fraction of antisera prepared by precipitation in the presence of 50% saturated ammonium sulfate (SAS) followed by dialysis against PBS for 48 hr.

Serum anti-morphine antibody binding activity was determined by precipitation of ^{14}C-morphine-antibody complexes by a modification of the ammonium sulfate method [15,21]. Duplicate 0.1 ml aliquots of serial dilutions of serum were mixed with 0.2 ml of solutions containing 100 pmole/ml ^{14}C-morphine (Amersham-Searle, Chicago, IL) and incubated overnight at 4°C. An equal volume of SAS was then added to each tube, and after incubation for 30 min, the tubes were centrifuged at 2500 rpm for 30 min at 4°C. The precipitates were washed in 50% SAS, solubilized and mixed with a toluene based scintillation fluid, and counted in a β scintillation counter. An antigen binding capacity-33 (ABC-33) was calculated from the antibody dilution capable of binding 33% of the radiolabeled antigen added and expressed as pmoles ^{14}C-morphine bound per ml of undiluted antiserum [15,21]. The ABC-33 reflects both serum antibody concentration and affinity (equilibrium association constant). The clearance of antigen-antibody complexes from the circulation may differ from either that of antigen or antibody alone [5, 11, 12]. Since M-6-HS-BSA contains bovine serum albumin (BSA) as carrier protein, animals immunized with this conjugate also developed circulating anti-BSA antibody. The presence of this second antibody permitted measurement of the effect of heroin binding on the clearance of anti-morphine antibody. The anti-BSA binding activity was determined in selected serum samples by the ammonium sulfate method [15]. Results were expressed as pmoles ^{125}I-BSA bound per ml of undiluted serum when 3.5 pmoles/ml ^{125}I-BSA was the antigen concentration employed in the assay.

Measurement of Drug Seeking Behavior

Two Rhesus monkeys weighing between 5 and 6 kg were housed in wooden cubicles and restrained by a stainless steel harness and spring arm with protected double lumen polyvinylchloride catheters which had been implanted in a major vein and passed subcutaneously to an exit point in the back [17]. The catheter was connected to drug reservoirs through peristaltic pumps. The cubicle was equipped with two boxes containing levers and stimulus lights, each associated with heroin or cocaine. The first monkey (4019) was trained to self-administer either heroin or cocaine on alternate days on a fixed ratio 10 (FR 10) schedule. Cocaine which was not specifically bound by the antibody was used to detect nonspecific changes in self-administration following the passive transfer. Alternating heroin and cocaine sessions resulted in considerable variability, so to improve day-to-day stability, the second animal (6058) was restricted to heroin self-administration on an FR 10 schedule.

During the 2 hr test session, the illumination of the overhead and lever stimulus lights signalled the availability of drug. Under all conditions studied, infusions were marked by the termination of the stimulus light and the appearance of a different colored light. Infusion volume was kept constant at 1 ml per infusion delivered at a rate of 6 ml per min. In Animal 4019, heroin was available at 6 μg/kg per infusion while cocaine was available at 200 μg/kg. Animal 6058 received a heroin dose of 12 μg/kg per infusion. Daily intake of heroin was approximately the same for both animals. When naloxone was administered prior to a session, animals were observed for signs of withdrawal before beginning the session. Programming was accomplished with electromechanical equipment in an adjoining room.

After stable self-administration behavior was established, 10 ml serum/kg was delivered slowly through a venous catheter in divided doses over 24 hr. Animal 6058 received either 200 ml of antiserum or normal monkey serum, while 4019 received 370 ml of antiserum.

RESULTS

Both animals achieved a stable level of drug self-administration following several weeks of conditioning, and neither animal developed physical dependence or tolerance to heroin. Both animals behaved in response to saline substitution, antagonist pretreatment and dose manipulation as previously reported [1,9]. To rule out involvement of other serum factors contributing to the behavioral effects seen with transfer of antiserum, one animal received an infusion of normal monkey serum. Substitution of saline for heroin in four consecutive sessions resulted in an initial increase followed by extinction of drug-seeking behavior (Fig. 1A). Pretreatment with naloxone (10 μg/kg) (Fig. 1B) or a 50% reduction in the amount of heroin given per infusion (Fig. 1C) resulted in an increase in drug seeking behavior (number of infusions) during several subsequent sessions. Passive administration of 200 ml of normal monkey serum resulted in transient decreases in the number of infusions (Fig. 1D).

The effect of different doses of naloxone on heroin self-administration resulted in an inverted U self-administration curve (Fig. 2). When given prior to several consecutive heroin sessions, doses of naloxone up to 100 μg/kg increased heroin self-administration whereas doses of 500 and 1000 μg/kg resulted in suppression of behavior. Naloxone pretreatment resulted in significant differences ($p<0.01$) from saline pretreatment for all doses excepting the 100 μg/kg which, as shown in Fig. 2, was the inflection point.

The effect of passive administration of anti-morphine antisera on heroin self-administration was measured in both experimental animals. Animal 6058 received 200 ml of whole anti-M-6-HS-BSA antiserum and results are shown in Fig. 3. The binding capacity of the administered antiserum was

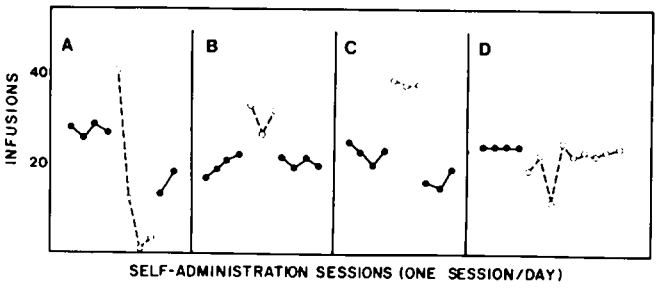

FIG. 1. Effect on infusions taken per two hr session by Monkey 6058: (A) substitution of saline for heroin; (B) pretreatment with 10 μg/kg naloxone; (C) lowering heroin dose from 12 μg/kg/infusion to 6 μg/kg/infusion; (D) administration of 200 ml of normal monkey sera. ●——● indicates heroin baseline and ○ – – ○ indicates respective experimental manipulations. A minimum of 10 days of stable self-administration behavior separated each manipulation.

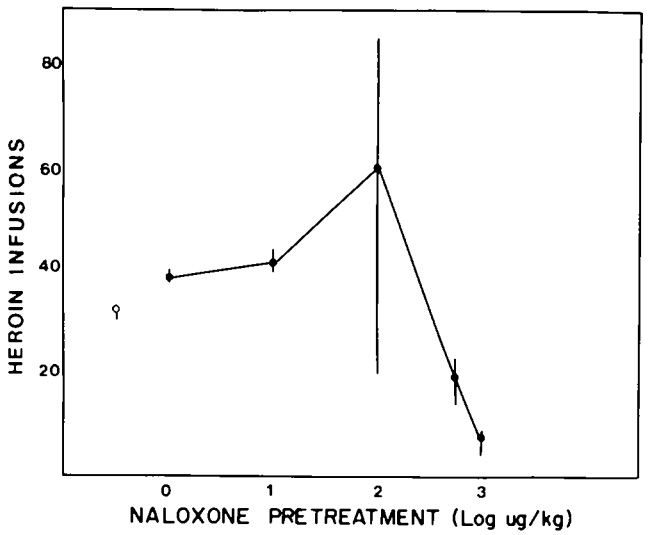

FIG. 2. The effect of varying doses of naloxone on heroin self-administration in Animal 4019. Naloxone was given before consecutive heroin and cocaine sessions for 2 to 3 weeks. Each point represents the mean effect of the last three days of naloxone administrations given prior to heroin sessions. The range is indicated by the vertical bars. The mean for saline is indicated by the open circle.

23,265 pmole ^{14}C-morphine per ml of undiluted serum. After equilibration of the globulin between the intra- and extravascular compartments (24 hr), the circulating antimorphine binding capacity in the recipient monkey was 8,184 pmole ^{14}C-morphine per ml. Immediately following completion of the serum transfer, the animal self-administered heroin at greater than 200% of baseline intake. With succeeding sessions, heroin self-administration gradually decreased and returned to baseline levels by 3 weeks. The rate of decrease in drug-seeking behavior closely paralleled the rate of decrease of the circulating anti-morphine activity suggesting that the observed change in behavior could be attributed to passive immunization. The rates of decrease of both anti-morphine and anti-BSA antibody activity were parallel suggesting that the clearance of the passively administered antimorphine

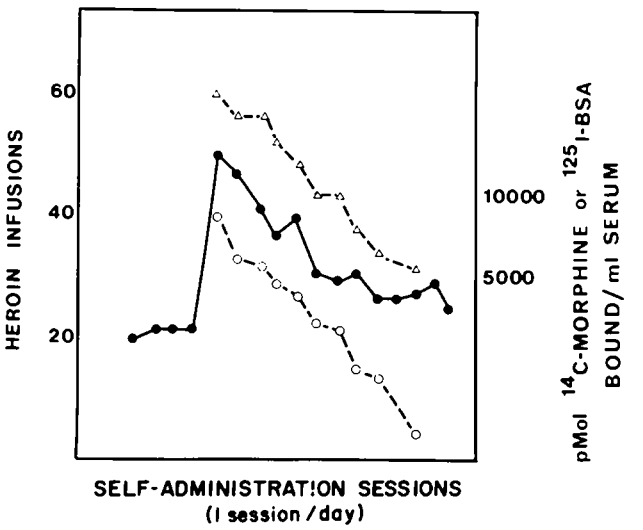

FIG. 3. Heroin self-administration per 2 hr session following passive administration of anti-M-6-HS-BSA antisera to Animal 6058: ●——● indicates infusions per session, ○ – – ○ indicates circulating anti-morphine binding activity expressed as pmole ^{14}C-morphine bound/ml serum, and △ – – △ indicates circulating anti-BSA antibody activity expressed as pmole ^{125}I-BSA bound/ml serum.

antibody was not altered by the presence of heroin. Administration of antiserum to Animal 4019 produced higher circulating antibody levels (20,790 pmole ^{14}C-morphine per ml) and heroin intake on the first day following transfer was 330% of baseline levels. No significant change in cocaine self-administration was observed following transfer demonstrating that the changes in heroin self-administration were immunologically specific. As in Monkey 6058, the decrease in daily heroin infusions following serum transfer paralleled the decline of circulating antibody activity. Typical cumulative records for Animal 4019 are shown in Fig. 4. The baseline heroin cumulative record (Fig. 4A) shows the highest frequency of lever-pressing responses and drug infusions at the beginning of the test session and a gradual tapering off in the second hr. Pretreating the animals with 100 μg/kg naloxone (Fig. 4B) causes a significant increase in responding, presumably to overcome the presence of the antagonist and achieve a pharmacologic effect. A similar increase in responses was observed when specific anti-morphine antibody was present in the circulation (Fig. 4C). In both instances, the increases were seen in the first half of the session. In contrast, the baseline response for cocaine self-administration (Fig. 4D) was not affected by either prior treatment with naloxone (Fig. 4E), or infusion of anti-morphine antibody (Fig. 4F).

Passive transfer of a crude globulin fraction of monkey anti-morphine antiserum was performed on Monkey 4019, and the behavioral response was similar to that observed with whole antiserum. A sample of cerebrospinal fluid was obtained by lumbar puncture in Animal 4019 following the whole serum transfer, and no circulating anti-morphine antibody activity was detected in the cerebrospinal fluid.

DISCUSSION

The results of the present experiments demonstrate that passive transfer of anti-morphine antibody resulted in a sig-

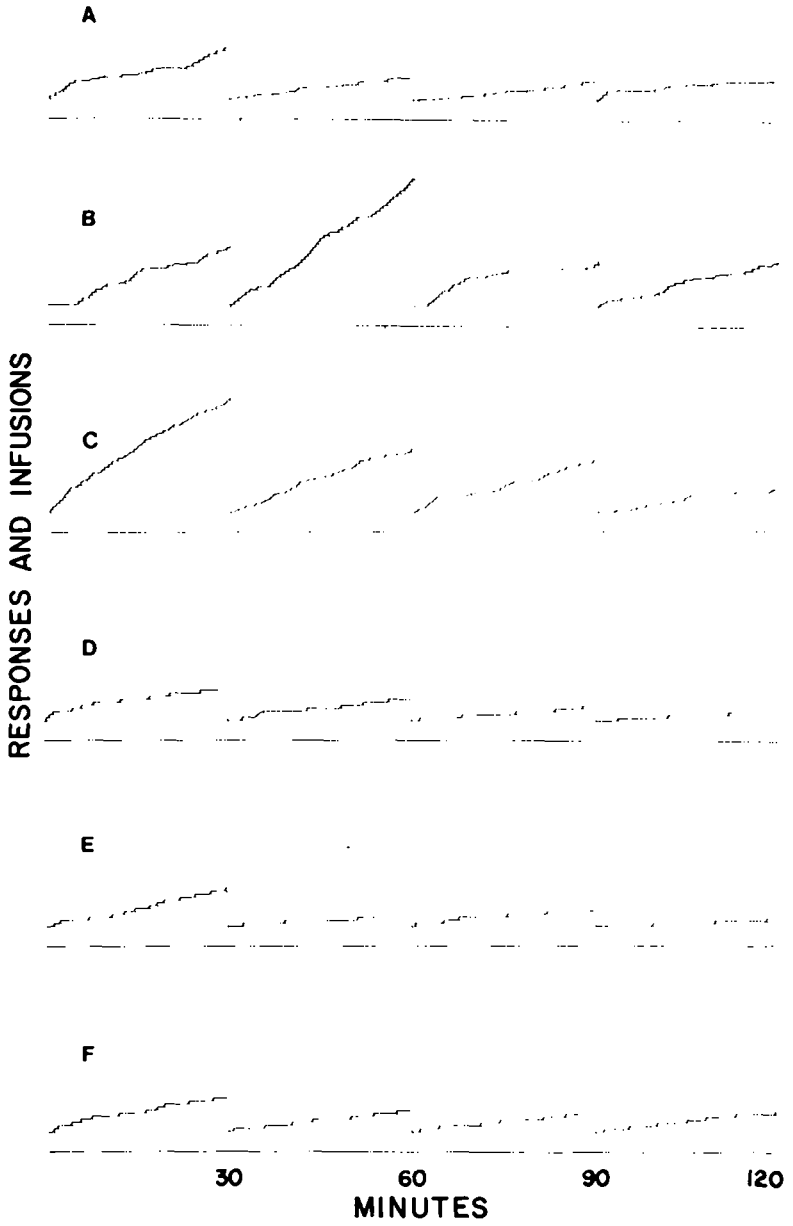

FIG. 4. Cumulative records of Monkey 4019 for heroin and cocaine self-administration. Record A is for a heroin self-administration control session; B, 100 μg/kg naloxone pretreatment; and C, transfer of whole antisera. D,E,F show cocaine records under identical conditions—control, naloxone pretreatment, and antisera transfer.

nificant increase in the amount of heroin self-administration by conditioned monkeys. The rate of decay of the circulating antibody activity in the recipient monkeys closely paralleled the return of the amount of heroin taken during each session to baseline levels and normal monkey serum did not produce changes in behavior. These findings are consistent with earlier studies demonstrating changes in heroin self-administration by a Rhesus monkey following active immunization against morphine [2], and suggest that the changes in behavior observed were mediated by specific immunologic mechanisms.

While these results generally confirm findings observed following active immunization, several differences are apparent. In the previous actively immunized animal, heroin self-administration was extinguished whereas in both passively immunized animals, heroin intake increased. This discrepancy may be explained by comparing the experimental protocols used. One factor may be the immunization-interval (approximately 20 weeks) imposed between initial shaping of drug self-administration behavior and reintroduction of drug following active immunization. When drug did again become available, the animal resumed

his former baseline of cocaine intake but failed to respond for heroin. The passively immunized monkeys in the present study were exposed to regular heroin self-administration sessions up through the time that passive antibody was given. Another difference is the concentration of circulating antibody activity achieved. The morphine binding capacity in the actively immunized animal reached 77,550 pmole ^{14}C-morphine per ml of serum whereas the highest concentration achieved in the passively immunized animals was one fourth of that. Further, whereas antibodies were found in the cerebrospinal fluid of the actively immunized monkey, none were found in the cerebrospinal fluid from one of the passively immunized animals. Normally, small concentrations of immunoglobulins are present in cerebrospinal fluid relative to serum [25]. Active immunization results in marked proliferation and changes in recirculation of lymphoid cells that affect the expression of cellular and humoral immunity both systemically as well as locally [16]. While passive immunization would be expected to result in circulating antibody activity in serum, significant amounts of antibody may not cross the blood-brain barrier. Active immunization, in contrast, has the capacity to stimulate local production of antibody in the central nervous system.

The behavioral response for opiates has been shown to vary inversely with dose until the dose per infusion is too small to support self-administration behavior at which point such behavior ceases [1]. The presence of circulating antibody presumably binds drug in the circulation and effectively lowers the concentration of heroin available to act at the central sites which mediate the reinforcing effects. In the actively immunized animal [2], high circulating levels of antibody probably bound most of the available opiate before reaching the brain. When the dose of heroin was increased to 16 times the original reinforcing dose, self-administration was again initiated. In the passively immunized animals with lower circulating levels of antibody, the heroin effect appeared to have been only partially antagonized resulting in an increase in the behavioral response. These effects are similar to that seen with the dose-related effects of naloxone. In the present study, low doses of naloxone resulted in an increase of heroin intake whereas the present and previous studies [9] have shown that large doses suppress self-administration behavior. Similar dose-related effects have been observed elsewhere in both nondependent and dependent animals [7,26].

In the present experiments, the rate of decay of circulating anti-morphine antibody activity did not differ significantly from that observed for anti-BSA. With large protein antigens such as BSA, administration of antigen to an immune animal results in enhanced clearance of both antibody and antigen [5, 11, 12]. Animals immunized with haptens such as morphine [8] or cardiac glycosides [3] have exhibited prolonged circulation of the hapten. The clearance of specific antihapten antibody has not been previously studied but the present data suggests that it is not significantly altered.

Specific antagonists of pharmacologic agents have provided valuable tools for studying mechanisms of drug action. These have been especially useful in the field of opiate pharmacology where antagonists such as naloxone are available. Results of the present and previous studies have shown that specific antibodies can effectively antagonize certain biologic actions of opiates [2, 13, 24]. Antibodies presumably exert their actions differently than the other narcotic antagonists and probably serve as a population of null receptors which effectively alter the equilibrium between free drug and drug molecules bound to pharmacologic receptors. While the present study has shown that both naloxone- and antibody-mediated antagonism of heroin self-administration behavior are quantitatively and qualitatively similar, one cannot assume that such parallels will be maintained in different pharmacological assays and under different physiological circumstances. Recent studies have shown a striking lack of parallelism in the effects of naloxone and anti-morphine antibodies on the myenteric plexus of tolerant-dependent guinea pigs [10]. Naloxone produces a characteristic contracture of the smooth muscle in such preparations which is in many ways analogous to precipitated abstinance [6]. While anti-morphine antibodies effectively reverse morphine depression of the electrically-stimulated twitch response, they do not elicit a naloxone-like contracture, nor do they attenuate the contracture evoked by a subsequent dose of naloxone. Similar studies can be extended to the behavior of animals which have been rendered tolerant and dependent to opiates and also to other pharmacological assay systems. Differences observed between the effects of narcotic antagonists and immunological antagonists, may lead to a clearer understanding of opiate actions at the receptor level.

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