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Antagonist Treatment in Nucleus Accumbens or Periaqueductal Grey Affects Heroin Self-Administration[1]

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CORRIGALL, W. A. AND F. J. VACCARINO. *Antagonist treatment in nucleus accumbens or periaqueductal grey affects heroin self-administration.* PHARMACOL BIOCHEM BEHAV 30(2) 443–450, 1988.—The role of opiate receptors in the periaqueductal grey and nucleus accumbens in maintenance of intravenous herion self-administration was examined by means of intracranial microinjections of the quaternary opiate antagonist methyl naltrexone. Over a dose range of 0–3.0 micrograms, pre-session infusions of methyl naltrexone in either brain site produced dose-related increases in responding for heroin (0.06 mg/kg/infusion) on a CRF schedule, without causing significant changes in responding on a second activity control lever. Involvement of the periaqueductal grey was also examined in animals administering a lower heroin dose (0.03 mg/kg/infusion) in shorter sessions in order to minimize drug exposure prior to treatment. In this experiment, infusion of methyl naltrexone produced selective increases in responding for heroin, whereas treatment with the identical dose of methyl naltrexone had no effect on cocaine self-administration (1.0 mg/kg/infusion) in the same animals. With respect to the nucleus accumbens, these data confirm its involvement in opiate self-administration. Data for the periaqueductal grey provide the first evidence that opiate receptors in the vicinity of this brain region may play a role in intravenous opiate self-administration.

Opiate self-administration Methyl naltrexone Periaqueductal grey Heroin Nucleus accumbens

WITH respect to the question of neuronal substrates of opiate reinforcement, a large body of evidence indicates that the neurons of the ventral tegmental area (VTA) are likely candidates. For example, it is known that conditioned place preferences occur after opiate microinjections into the VTA [18,19], extinguished operant self-administration behavior can be reinstated by non-contingent brain microinfusions of opiate agonists into the VTA [24], self-administration behavior is maintained with intracranial agonist infusions into the VTA [5], and intravenous heroin self-administration is altered by microinfusions of opiate antagonist into the VTA [1]. Dopaminergic neurons in the VTA have also been implicated in reinforcement through studies showing that conditioned place preference produced by systemic opiates or VTA microinjections are sensitive to haloperidol [20], pimozide [4] and 6-hydroxydopamine lesions at the level of the nucleus accumbens [23], and by the finding that morphine self-administration is increased by lesions of the nucleus accumbens [22].

In addition to the above evidence, however, other brain sites have been implicated in opiate-produced place conditioning [28] and in opiate self-administration [6, 8, 14–16]. In addition, the role of dopamine in opiate reward has not been supported by several recent investigations using place preference [13] and self-administration [7,17] techniques. Therefore, there is at present no clear understanding of the brain substrates which underlie opiate reinforcement.

To address this issue, we have begun to examine brain sites, both within and separate from the mesolimbic dopamine system, in order to determine whether they participate in maintenance of opiate self-administration behavior. The approach that we have used is to produce local antagonism in the brain sites of interest by means of central microinjection of a quaternary opiate antagonist and to measure the effect of this treatment on intravenous heroin self-administration. The antagonist that we have used in this research is methyl naltrexone, since it provides the best combination of reduced lipophilicity while retaining potency at the opiate receptor.

We report here the effects of treatments with this

[1]The views expressed in this publication are those of the authors and do not necessarily reflect those of the Addiction Research Foundation.

antagonist in the periaqueductal grey (PAG) and the nucleus accumbens (ACC) on intravenous heroin self-administration. We chose to examine the ACC since its involvement in opiate reinforcement has been the object of some controversy. For example, one study has reported that the ACC will not support intracranial self-administration [3], while others have indicated that it will [8,15]. Two previous studies using different quaternary antagonists than methyl naltrexone have reported divergent findings with respect to the effects of intra-ACC injections of these antagonists on intravenous opiate self-administration [1,27]. However, both of these studies employed quaternary antagonists which retain substantial lipid solubility compared to methyl naltrexone [2]. Therefore, to clarify whether or not the ACC does participate in mediating opiate self-administration, it was necessary to re-examine this structure using an antagonist which does not spread readily through lipid.

We chose to examine the PAG since agonist microinjections within this structure have been shown to produce a conditioned place preference [28], and secondarily because the PAG may have a role in mediating the discriminative stimulus properties of opiates [12], a feature which might be related to self-administration.

METHOD

Three distinct experimental groups form the basis of this report. One group constituted a pilot experiment to establish the time course of acquisition of heroin self-administration, and to determine dose parameters for methyl naltrexone in subsequent experiments. The first quantitative experiment (Experiment 1) was an assessment of the effects of methyl naltrexone pretreatment in the ACC and the PAG on intravenous heroin self-administration. The second, Experiment 2, was a replication of the findings for the PAG with animals administering a lower dose of heroin in shorter sessions, and in addition included an examination of whether the antagonist effects were specific for opiate as compared to stimulant self-administration.

Subjects in all cases were male Long-Evans rats (Charles River, Lachine, Quebec), drug naive at the start of the experiment. Animals were housed in a reversed light-dark cycle (lights off between 7:00 and 19:00 hours), and were allowed to reach approximately 300 g in weight before training procedures were begun.

Initial training consisted of shaping the animals to respond on a CRF schedule for food reinforcement (45 g Noyes pellet) while maintained on restricted access to food (to keep body weights approximately 85% of free-feeding values). Once the animals had been trained to lever press, they were returned to ad lib access to food for the duration of the experiment.

Each animal was then surgically prepared under pentobarbital anesthesia (60 mg/kg, IP) with a chronic intravenous catheter in the jugular vein. Catheter construction was the same as previously described [6]. During the initial recovery period from surgery (3–7 days), catheters were flushed once each day with 0.1 ml sterile saline containing heparin (5 units USP/ml). Drug self-administration sessions were begun after this recovery period. During self-administration, catheters were flushed with sterile saline once daily prior to the operant session; catheters were not flushed on weekends. At all times when catheters were not in use, external ends were plugged with obturators to prevent back flow of blood and entry of foreign material. Patency of catheters was assessed periodically by means of methohexital infusion (3–5 mg/kg).

Self-administration sessions were carried out in dual-lever operant chambers. Experimental control and data acquisition were performed by a Pascal-based system operating an IBM-PC microcomputer [11]. Drug was available on a CRF schedule during a single session each weekday. In the pilot experiment (n=14) and Experiment 1 (n=16) heroin hydrochloride was available at a dose of 0.06 mg/kg/infusion and a session duration of 3 hours. In Experiment 2 the session duration was 90 minutes; during this time animals had access to either heroin hydrochloride at a dose of 0.03 mg/kg/infusion or cocaine hydrochloride at a dose of 1.0 mg/kg/infusion. In this latter experiment, animals acquired self-administration behavior with either cocaine (n=4) or heroin (n=7), underwent brain surgery and antagonist treatment as described below, and were then switched to receive the other drug; when their baseline responses were again stable, treatment was repeated. In all groups, drug delivery (100 microliters/kg) occurred during a one-second period following a response on the appropriate lever. Responses on the drug lever (denoted as DRUG in the following discussion) were followed by a 5-second time-out period, signalled by a buzzer, during which additional responses (denoted as TIME-OUT responses) were recorded but not reinforced. Similarly, responses on the non-drug lever (denoted as INACTIVE) had no consequences for the animal, but were recorded.

Following acquisition, animals were implanted with bilateral brain cannulae positioned stereotaxically according to the atlas of König and Klippel [10]. Commercial guide cannulae (Plastic Products, Roanoke, VA) were cut prior to surgery so that they would extend no closer than 1 mm to the target site. This surgery was also performed under pentobarbital anesthesia (60 mg/kg, IP), with the animal positioned in a stereotaxic frame such that the top of the skull overlying the cortex was horizontal. The following coordinates were used: ACC, +2.4 mm relative to bregma, +3.0 mm lateral to midline, angled 17 degrees from the midline plane; PAG, −6.2 mm relative to bregma, +2.5 mm lateral to midline, angled 18 degrees from the midline plane; VTA (pilot animals only), −5 mm relative to bregma, +3.0 mm lateral to midline, angled 16 degrees from the midline plane. At the conclusion of the experiment, animals were given an overdose of pentobarbital and perfused with saline followed by 10% formalin. Brains were removed and prepared for histology to confirm cannulae placement.

Brain microinjections were carried out as previously described [6]. Microinjections of the antagonist or saline vehicle were given bilaterally 10 minutes prior to the beginning of treatment sessions. Injection volume was constant at 0.5 microliter per unilateral site. Brain injections were delivered by means of two gas-tight microsyringes each driven by a manually-operated micrometer screw; the micrometers were advanced alternately in small increments. The volume of antagonist delivered was determined by monitoring movement of a small bubble placed in calibrated polyethylene tubing between the microsyringes and the rat. Micrometer screws were advanced intermittently so as to cause the air bubble to move through 0.5 microliter volume of the tubing in approximately 2 minutes.

Based on observations in the pilot animals (see below), doses of methyl naltrexone of 0, 0.1, 0.3, 1.0, and 3.0 micrograms total dose were chosen for use in Experiment 1. In this experiment, treatments were done with ascending doses;

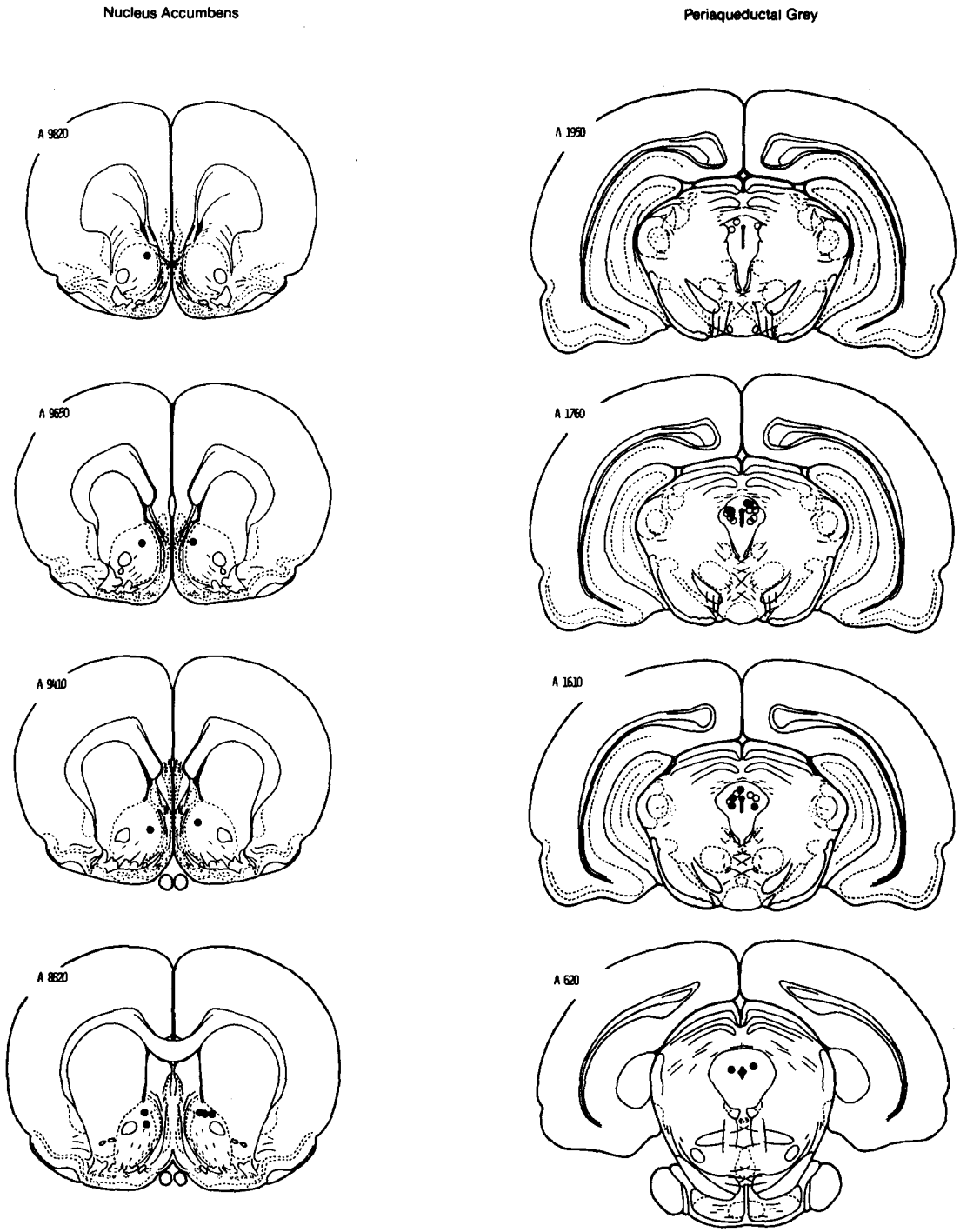

FIG. 1. Location of cannulae tips of the nucleus accumbens and periaqueductal grey in Experiment 1 (filled circles) and in the periaqueductal grey in Experiment 2 (open circles). Schematic brain sections have been adapted from the stereotaxic atlas of König and Klippel [10].

treatment days were separated by a minimum of 2 non-treatment days during which animals had access to heroin without any treatment. This scheduling was chosen on the basis of pilot data which showed that the effect of the antagonist on drug intake (i.e., the DRUG measure, the main focus of the research) was virtually absent by the day following treatment. In addition to receiving brain microinjections of antagonist, animals in Experiment 1 also received an initial treatment with naltrexone (0.3 mg/kg IV) given 2 minutes prior to the start of the session. This treatment was to permit assessment of the effect of general systemic antagonism. In Experiment 2, animals were treated with only 0 and 1.0 mi-

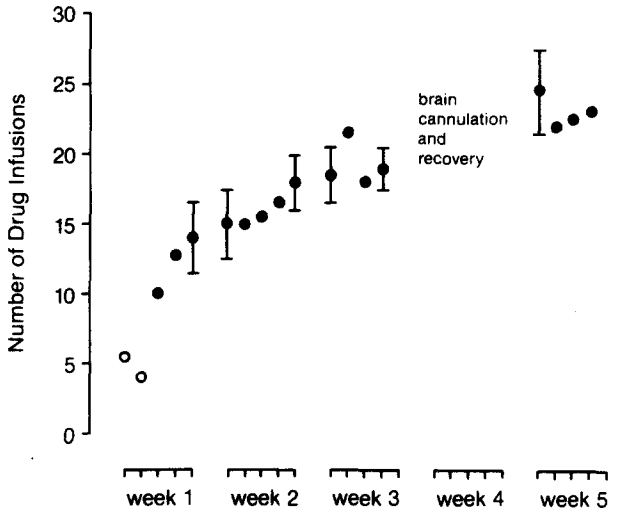

FIG. 2. Data from pilot experiment. Acquisition of responding for heroin (0.06 mg/kg/infusion) on a CRF schedule in 3-hour sessions. Animals are given drug-free weekends. Brain cannulation and recovery occurred during the fourth week. Data are means for the pilot group (n=14); where shown, error bars are ±1 standard error of the mean. The first two data points (open circles) are from shorter than 3-hour sessions.

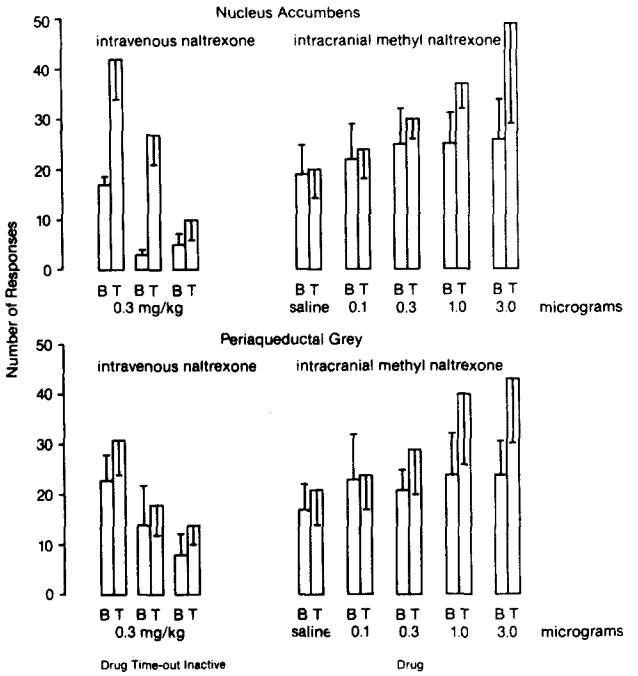

FIG. 3. Data from Experiment 1. Values for DRUG, TIMEOUT, and INACTIVE responses in baseline (B) and intravenous naltrexone treatment (T) sessions in ACC and PAG animals (left hand side), and values for DRUG responses in baseline (B) and intracranial methyl naltrexone treatment (T) sessions across 5 doses (right hand side). Baseline sesions are those on the day immediately preceding the treatment session. Data are means for Group 1 (for ACC, n=7 for intravenous naltrexone, n=5 for intracranial methyl naltrexone; for PAG, n=9 for intravenous naltrexone, n=4 for intracranial methyl naltrexone); error bars show ±1 standard error of the mean.

crograms of methyl naltrexone, rather than the more extensive dose regimen used in Experiment 1; however, they received these treatments twice, once when responding for 0.03 mg/kg heroin and once when responding for 1.0 mg/kg cocaine.

It is not uncommon in opiate self-administration for the drug intake to continue to rise gradually over days, even long after acquisition has occurred (e.g., see [25]). Therefore, to ensure that treatment effects were referenced to the most immediate non-treatment data, the non-treatment sessions immediately preceeding each treatment session were used in analyses; these non-treatment sessions are referred to as baseline sessions.

Statistical treatment of data from Experiment 1 was by analysis of variance of the differences between logarithmically transformed baseline and treatment session values for each of DRUG, TIME-OUT and INACTIVE measures. The logarithmic transformation was done because we noted that the distribution was long-tailed towards high values, a common occurrence when data are of the nature of counts and particularly in the case of opiate self-administration (see for example the distribution of responding in [25]). Because analyses were done on the difference scores, the analyses of variance are reduced by one factor; that is, rather than testing for a treatment effect against a baseline, we are testing the difference value for dose and site effects. Data from Experiment 2 were analyzed by *t*-tests.

RESULTS

Cannulae placements from both experiments are shown in Fig. 1. For all subjects, cannulae were located anatomically within the ACC or PAG, and no subjects were rejected on the basis of cannulae placement.

Drug intake in the pilot group both during acquisition and after brain cannulation is shown in Fig. 2. In some of these pilot animals we tested the effects of doses of 0.1 and 1.0 micrograms of methyl naltrexone on heroin self-administration, and found that the lower dose appeared to be below threshold for producing changes, but that the dose of 1.0 microgram was effective in either ACC or VTA. In other pilot animals we tested a dose of 10 micrograms in the VTA, but found that when infused prior to a self-administration session it produced seizures within several minutes.

In Experiment 1, sixteen animals which acquired heroin self-administration similarly to the pilot animals were cannulated as described above (ACC, n=7; PAG, n=9) and returned to self-administration. Figure 3 (left-hand side) shows the results of pre-session treatment of these animals with 0.3 mg/kg naltrexone IV. Naltrexone caused an increase in responding for intravenous heroin, as has been observed in other studies in which antagonists have been given systemically to animals self-administering opiate agonists (e.g., [6, 9, 27]). One-way analysis of variance showed that there was no significant effect of brain site in the DRUG, $F(1,14)=1.82$, $p=0.20$, or INACTIVE measures, $F(1,14)=0.81$, $p=0.38$, and the effect of brain site reached borderline significance in the TIME-OUT measure, $F(1,14)=4.46$, $p=0.05$. Overall, therefore, both groups were statistically similar in their response to systemic naltrexone.

For each of the ACC and PAG squads, at least six animals were treated at each dose of centrally-administered antagonist to the end of the experiment. However, in a few

TABLE 1

MEAN BASELINE AND INTRACRANIAL ANTAGONIST TREATMENT SCORES FOR RESPONDING DURING TIME-OUT AND ON THE INACTIVE LEVER

Site and Measure	Dose									
	Saline		0.1 Microgram		0.3 Microgram		1.0 Microgram		3.0 Microgram	
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
ACC (n=5)										
Inactive	6.8 (3.4)	12.6(12.2)	4.6 (1.5)	4.2 (2.6)	4.4 (1.6)	4.8 (2.3)	5.2 (2.6)	7.6 (2.9)	4.6 (2.2)	6.4 (3.6)
Time-Out	8.8 (7.2)	12.8 (8.9)	11.8(10.8)	12.0(11.0)	18.2(11.3)	16.6 (9.5)	14.6(13.3)	19.2(10.3)	13.8(11.9)	57.6(53.3)
PAG (n=4)										
Inactive	17.3(17.6)	8.0 (4.0)	6.8 (3.6)	39.5(34.5)	26.5(25.8)	8.0 (2.0)	6.5 (4.9)	13.8(12.7)	3.0 (1.4)	32.0(34.5)
Time-Out	11.5(12.4)	20.3(21.5)	20.0(21.6)	19.5(19.1)	9.3 (3.8)	25.8(21.8)	16.3(14.4)	32.0(28.6)	16.0(15.7)	24.5(21.0)

cases animals had been substituted for others missing due to attribution (because of loss of patency of intravenous catheters or physical damage to brain cannulae). In the ACC there were 5 animals which had completed all treatments, while in the PAG there were 4; this subset of the full data set was chosen for analysis so as to be able to use a repeated measures design. Figure 3 (right-hand side) shows that data for the DRUG response across doses of methyl naltrexone in the two sites; Table 1 lists the values for TIME-OUT and INACTIVE measures. For the DRUG response, analysis of variance showed no significat brain site × antagonist dose interaction, $F(4,28)=0.17$, $p=0.95$, a significant effect of antagonist dose, $F(4,28)=6.02$, $p<0.001$, and no effect of brain site, $F(1,7)=0.25$, $p=0.63$.

The small increases in DRUG responses over the experiment deserves comment. As noted above, it is not unusual for a gradual but sustained increase in opiate intake to occur over days. Since brain anatagonist treatments were carried out over a period of several weeks, it is not surprising that the non-treatment sessions showed small increases. These increases are much smaller than the effect produced by antagonist microinjection. Furthermore, they are taken into account in analysis, since it is the difference between treatment and baseline measures which are used.

For the TIME-OUT response there was again no site × dose interaction, $F=1.32$, $p=0.29$, a significant dose effect, $F(4,28)=4.33$, $p<0.01$, and no effect on brain site, $F(1,7)=0.06$, $p=0.81$. For the INACTIVE response, there was no site × dose interaction, $F(4,28)=0.34$, $p=0.85$, and no dose, $F(4,28)=0.33$, $p=0.86$, or site, $F(1,7)=1.16$, $p=0.32$, effects.

Results of methyl naltrexone pre-treatment on both heroin and cocaine responding in Experiment 2 are shown in Fig. 4. Intra-PAG injections of saline were without effect on either cocaine or heroin self-administration (Fig. 4b). Similarly, in the case of cocaine-reinforced behavior, intra-PAG injections of methyl naltrexone at a dose of 1 microgram clearly did not alter responding on the drug lever, as compared to the respective baseline values. In the case of heroin-maintained responding, however, methyl naltrexone pre-treatment with 1.0 microgram produced a significant increase in drug-lever responding during normal access periods compared to the previous non-treatment session, $t=2.42$, $p<0.05$, as well as a significant increase in time-out responding, $t=2.48$, $p<0.05$; responding on the inactive lever was unaffected. Note that in contrast to the effect of the antagonist on heroin self-administration, both the rate and the pattern of cocaine intake were unaffected (Fig. 4a).

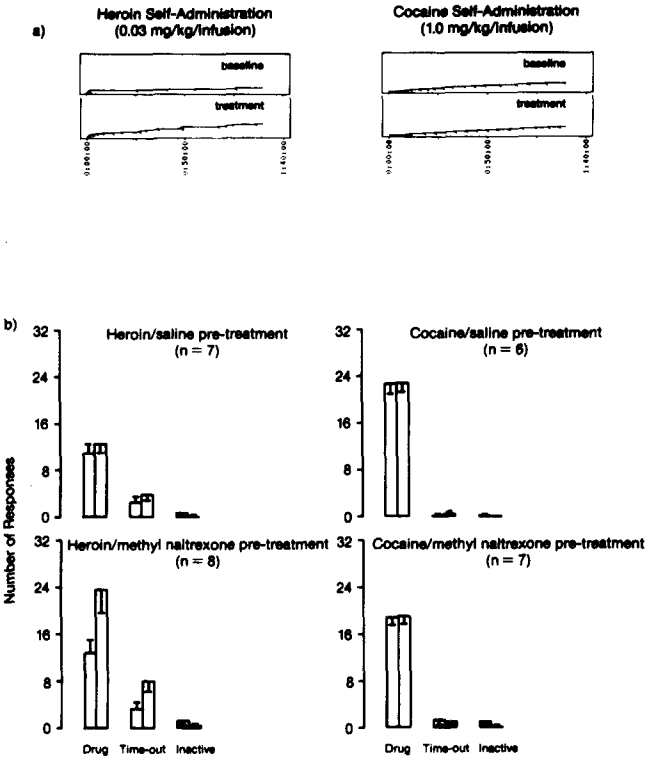

FIG. 4. Data from Experiment 2. (a) Examples of cumulative records for one subject during baseline and treatment (1.0 microgram methyl naltrexone) sessions in heroin self-adminstration (left hand side), and records for the same animal, approximately 2 weeks later, during identical treatment in cocaine self-administration session. (b) Values for DRUG, TIME-OUT, and INACTIVE responses in baseline (B) and treatment (T), comparing effects of intra-PAG saline or antagonist injections on heroin (0.03 mg/kg/infusion) and cocaine (1.0 mg/kg/infusion) responding. Baseline sessions are those on the day immediately preceeding the treatment session. Error bars are ±1 standard error of the mean.

DISCUSSION

From observations in the pilot group, acquisition of heroin self-administration, defined as the period required for drug intake to reach a relative plateau, occurred in approximately two weeks (e.g., results shown in Fig. 2). In conse-

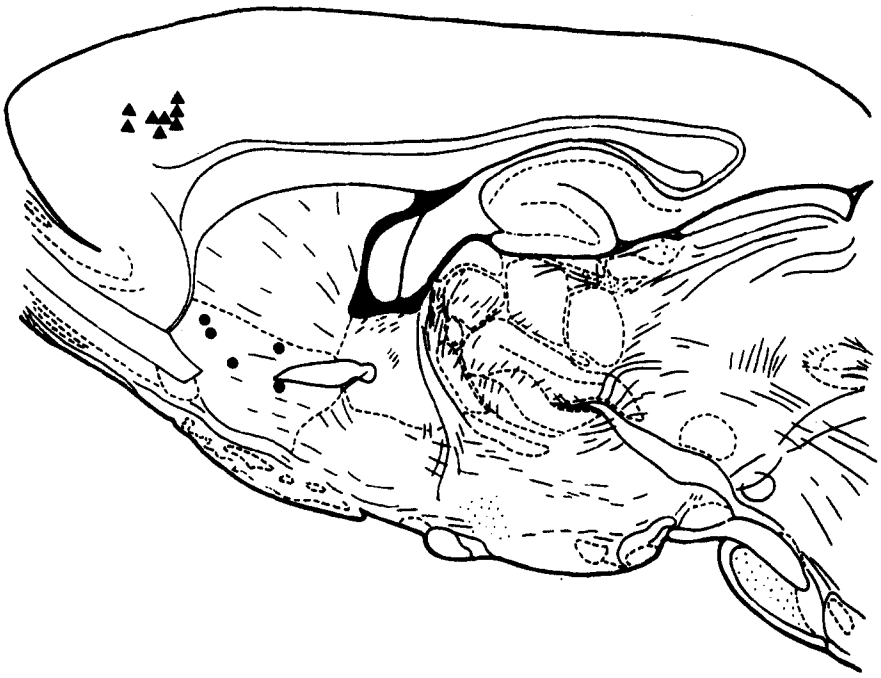

FIG. 5. Schematic illustration in sagittal view of cannulae tips in ACC from this study (circles) at which methyl naltrexone was effective in altering heroin self-administration, and from medial prefrontal cortex sites (triangle) at which methyl naltrexone was completely ineffective. Brain section was adapted from the atlas of König and Klippel [10].

quence, a baseline of stable drug intake was available after a relatively short period of heroin exposure. On this baseline, low-dose methyl naltrexone microinjections (of the order of 1 microgram) into either the ACC or VTA produced increases in heroin self-administration similar to those described following treatment with other quaternary antagonists, whereas at high doses (10 micrograms), seizures were produced. Seizure-induction may be a general characteristic of high doses of quaternary antagonists; for example, methyl naloxone produces tremors and convulsions at a dose range of 10–30 micrograms intracerebroventricularly [2], and in a recent study examining opioid mechanisms in water intake, methyl naltrexone has been reported to produce effects such as rotational behaviors and convulsions in a dose- and brain site-dependent fashion [26]. These observations suggest that one must use care in choosing not only the particular quaternary antagonist but also the dose range to employ. Indeed it was reasons such as these that led us to do a pilot experiment to assess the dose range of methyl naltrexone to use in this study; findings in that experiment resulted in choice of the 0.1–3.0 microgram dose range for methyl naltrexone.

When challenged with naltrexone systemically, animals in Experiment 1 showed an increase in drug taking itself, as well as continued responding on the drug lever during the time-out period, both of which can be interpreted as a compensatory response of the animals to the decreased effectiveness of the reinforcer produced by the antagonist. The dose-dependent increase in responding on the drug lever (in both the DRUG and TIME-OUT measures) after central methyl naltrexone pre-treatments is subject to the same interpretation as the increase seen after systemic naltrexone, namely, a compensatory increase in drug taking by the animals to offset decreased efficacy of the reinforcer, in this case produced as a result of antagonist action locally in the brain. This data suggests that each of ACC and PAG have a role in maintenance of herion self-administration.

The alterations in self-administration produced by central antagonist treatment cannot be accounted for simply on the grounds that the antagonists produced non-specific or withdrawal-based increases in responding for several reasons. First, the effect was observed in two separate groups of animals, at least one of which had had minimal heroin exposure at the time of testing. Also, during the 10 minute period between antagonist pre-treatment and the beginning of the heroin self-administration session, behaviors that could be ascribed to antagonist treatment were not generally observed. In addition, the antagonist itself was clearly not producing non-specific effects, since cocaine self-administration was completely unaffected by the same dose.

It is important to note that while intra-PAG or intra-ACC microinjections of methyl naltrexone result in increased self-administration of heroin, the contribution of receptors outside but in the vicinity of these sites cannot be ruled out in this study. Cannulae tips were within the anatomical boundaries of the respective sites, and therefore we do not have evidence as yet as to whether the effective opiate receptors are distributed exclusively within each site or beyond as well. However, a previous study from this laboratory has shown that methyl naltrexone injections into the medial prefrontal cortex (a few millimeters away from the nucleus accumbens) do not cause any change in heroin self-administration [6]; Fig. 5 shows schematically the histological distribution of these ineffective sites in relation to the positive accumbens sites reported here. The observation that there are negative sites within a few millimeters of the accumbens

sites suggests that methyl naltrexone remains relatively localized after brain injection, or that if it does diffuse, its concentrations rapidly become too small to be effective. In addition, it is notable that the distance between the accumbens and the medial prefrontal cortical sites is approximately comparable to the distance between the PAG and the VTA. It is unlikely, then, that effects seen following intra-PAG microinjections of methyl naltrexone are due to spread of the antagonist to the VTA.

Regarding this same point, other research has also provided evidence that methyl naltrexone does not spread throughout the brain. For example, doses in the range of 0.3 to 10 micrograms have been shown to be effective in reducing post-deprivational water intake when administered into the paraventricular hypothalamic nucleus, but not when administered into the lateral hypothalamus which is immediately adjacent [26].

Although the question of the distribution of receptors involved in reinforcement in the immediate vicinity of the ACC and PAG, and indeed around the VTA, is one which warrants future attention, the purpose of the present research was to locate general sites in the brain which appear to be involved in regulation of opiate self-administration as a focus to further studies. What is clear at present is that the PAG and ACC are each sites at which methyl naltrexone microinjections are effective in altering heroin self-administration.

With respect to the ACC, the results of this study support other observations that opiate receptors in and/or around this area of the brain are involved in heroin self-administration [8, 15, 27]. Beyond providing confirmation of the role of receptors in the vicinity of the ACC, however, this research suggests that opiate receptors in the PAG area are involved in maintenance of heroin self-administration. Other evidence relative to the role of opiate receptors in the PAG region in opiate reinforcement is minimal and indirect, and if marshalled, both supports and counters such a proposal. In a positive sense, opiate agonist injections into the PAG have been reported to produce a conditioned place preference [28], although the relationship between the latter phenomenon and self-administration remains unclear at present. Bozarth [3] has found that intracranial self-administration was not established by injections into the PAG, or for that matter, into the ACC. In addition, in a study of reinstatement of self-administration produced by central agonist treatments, Stewart [24] reported that intra-PAG agonist infusions were ineffective as compared to those in the VTA. It may be, however, that there are different systems involved in reinstatement of extinguished responding and maintenance of self-administration.

Some reconciliation of the divergent findings from various studies is possible, although speculative. Perhaps different brain areas subserve different affective features of the drug. For example, with respect to the PAG, there has been one suggestion that it has a role in morphine drug discrimination ([12], but see also [22]). It may be that antagonist treatment in the PAG alters self-administration because of an effect on the general drug discrimination stimulus complex. If that were the case, intracranial self-administration might not be obtained with intra-PAG agonist infusions, as in fact reported by Bozarth [3], but intravenous self-administration might be changed if intra-PAG antagonist treatment alters some affective feature of the drug such as the discrimination stimulus complex. In other words, the cues used when a drug is self-administered intravenously may be very different from those used when it is taken intracranially. If another site were responsible for the incentive properties, that site might support intracranial self-administration and in addition demonstrate alterations in intravenous agonist self-administration when subjected to brain microinjections. The VTA and ACC might be examples of such sites. What contribution, and by what mechanism, each site so far identified makes to opiate consumption is clearly an area deserving further investigation.

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REFERENCES

1. Britt, M. D. and R. A. Wise. Ventral tegmental site of opiate reward: antagonism by a hydrophilic opiate receptor blocker. *Brain Res* **258**: 105–108, 1983.
2. Brown, D. R. and L. I. Goldberg. The use of quaternary narcotic antagonists in opiate research. *Neuropharmacology* **24**: 181–191, 1985.
3. Bozarth, M. A. Opiate reward mechanisms mapped by intracranial self-administration. In: *The Neurobiology of Opiate Reward Processes*, edited by J. E. Smith and J. D. Lane. Amsterdam: Elsevier Biomedical Press, 1983, pp. 331–359.
4. Bozarth, M. A. and R. A. Wise. Heroin reward is dependent on a dopaminergic substrate. *Life Sci* **29**: 1881–1886, 1981.
5. Bozarth, M. A. and R. A. Wise. Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci* **28**: 551–555, 1981.
6. Corrigall, W. A. Heroin self-administration: Effects of antagonist treatment in the lateral hypothalamus. *Pharmacol Biochem Behav* **27**: 693–700, 1987.
7. Ettenberg, A., H. O. Pettit, F. E. Bloom and G. F. Koob. Heroin and cocaine intravenous self-administration in rats: Mediation by separate neural systems. *Psychopharmacology (Berlin)* **78**: 204–209, 1982.
8. Goeders, N. E., J. D. Lane and J. E. Smith. Self-administration of methionine enkephalin into the nucleus accumbens. *Pharmacol Biochem Behav* **20**: 451–455, 1984.
9. Goldberg, S. R., J. H. Woods and C. R. Schuster. Nalorphine-induced changes in morphine self-administration in rhesus monkeys. *J Pharmacol Exp Ther* **176**: 464–471, 1971.
10. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: Williams and Wilkins, 1963.
11. Kaplan, H. L. Design decisions in a Pascal-based operant conditioning system. *Behav Res Methods Instr Comput* **17**: 307–318, 1985.
12. Krynock, G. M. and J. A. Rosecrans. Morphine as a discriminative stimulus: role of periaqueductal gray neurons. *Res Commun Chem Pathol Pharmacol* **23**: 49–60, 1979.
13. Mackey, W. B. and D. van der Kooy. Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. *Pharmacol Biochem Behav* **22**: 101–105, 1985.
14. Olds, M. E. Hypothalamic substrates for the positive reinforcing properties of morphine in the rat. *Brain Res* **168**: 351–360, 1979.

15. Olds, M. E. Reinforcing effects of morphine in the nucleus accumbens. *Brain Res* **237**: 429–440, 1982.
16. Olds, M. E. and K. N. Williams. Self-administration of D-ala-met-enkephalinamide at hypothalamic self-stimulation sites. *Brain Res* **194**: 155–170, 1980.
17. Pettit, H. O., A. Ettenberg, F. E. Bloom and G. F. Koob. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology (Berlin)* **84**: 167–173, 1984.
18. Phillips, A. G. and F. G. LePiane. Reinforcing effects of morphine microinjections into the ventral tegmental area. *Pharmacol Biochem Behav* **12**: 965–968, 1980.
19. Phillips, A. G. and F. G. LePiane. Reward produced by microinjection of (D-Ala), Met-enkephalinamide into the ventral tegmental area. *Behav Brain Res* **5**: 225–229, 1982.
20. Phillips, A. G., F. G. LePiane and H. C. Fibiger. Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. *Life Sci* **33**: 2505–2511, 1983.
21. Shannon, H. E. and S. G. Holtzman. Discriminative effects of morphine administered intracerebrally in the rat. *Life Sci* **21**: 585–594, 1977.
22. Smith, J. E., G. F. Guerin, C. Co, T. S. Barr and J. D. Lane. Effects of 6-OHDA lesions of the central medial nucleus accumbens on rat intravenous morphine self-administration. *Pharmacol Biochem Behav* **23**: 843–849, 1985.
23. Spyraki, C., H. D. Fibiger and A. G. Phillips. Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. *Psychopharmacology (Berlin)* **79**: 278–283, 1983.
24. Stewart, J. Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. *Pharmacol Biochem Behav* **20**: 917–923, 1984.
25. Thompson, T. and R. Pickens. Stimulant self-administration by animals: some comparisons with opiate self-administration. *Fed Proc* **29**: 6–12, 1970.
26. Ukai, M. and S. G. Holtzman. Suppression of deprivation-induced water intake in the rat by opioid antagonists: central sites of action. *Psychopharmacology (Berlin)* **91**: 279–284, 1987.
27. Vaccarino, F. J., F. E. Bloom and G. F. Koob. Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. *Psychopharmacology (Berlin)* **86**: 37–42, 1985.
28. van der Kooy, D., R. F. Mucha, M. O'Shaughnessy and P. Bucenieks. Reinforcing effects of brain micro-injections of morphine revealed by conditioned place preference. *Brain Res* **243**: 107–117, 1982.