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Heroin Self-Administration: Effects of Antagonist Treatment in Lateral Hypothalamus[1]

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CORRIGALL, W. A. *Heroin self-administration: Effects of antagonist treatment in lateral hypothalamus.* PHARMACOL BIOCHEM BEHAV 27(4) 693–700, 1987.—The involvement of the lateral hypothalamus and medial prefrontal cortex in mediating heroin 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. In animals trained to respond on a continuous reinforcement schedule for intravenous heroin (0.03 mg/kg/infusion), microinfusions of antagonist into the lateral hypothalamus prior to a self-administration session produced significant dose-related increases in responding on the drug manipulandum, similar to increases in responding observed after treatment with naltrexone systemically. Microinfusions of quaternary antagonist into the medial prefrontal cortex over the same dose range effective in the lateral hypothalamus did not produce response increases. These data suggest that opiate action in the lateral hypothalamus, but not in the medial prefrontal cortex, is salient in maintenance of intravenous self-administration.

Opiate self-administration Lateral hypothalamus Medial prefrontal cortex Methyl naltrexone Heroin Extinction

ONE of the basic goals of opiate abuse research is the identification of the brain substrates involved in drug-taking behavior. A variety of strategies have been brought to bear on this question, including pharmacological [6] and electrolytic lesions [8], direct intracranial self-administration [2], and measurement of neurotransmitter changes correlated with consumption [14]. Another approach which is related to the same goal has been to identify the brain sites at which opiate binding is directly involved in self-administration behavior. The advantages of this approach is that it is likely to identify brain sites which are the first stage of the "reinforcement system." That is, this method should identify sites at which opiates bind to initiate processes which regulate their intake.

Several studies have used brain microinjections of quaternary opiate antagonists as a tool to identify such sites. As a result it is known that quaternary opiate antagonists alter intravenous self-administration when microinjected into the ventricles [16], the ventral tegmental area [4,15], and the nucleus accumbens [15]. In a recent study we confirmed the involvement of the nucleus accumbens, and demonstrated that, in addition, the periaqueductal grey region is involved in maintenance of low-dose, limited-access heroin self-administration (Corrigall and Vaccarino, manuscript in review).

While it may not be practical to examine each of numerous candidate brain sites, at least one additional brain region is deserving of attention, namely, the hypothalamus. Some years ago Kerr [10] proposed that the hypothalamus was directly involved as a site of action for opiates in mediating reinforcement. This was subsequently tested by Olds [12] and Olds and Williams [13] who reported that opiate agonists delivered directly into the posterior lateral hypothalamus maintained lever pressing behavior. However, these two studies were subsequently called into question on the grounds that the subjects had been previously trained to respond for electrical brain stimulation [2]. In addition, kainic acid lesions of the lateral hypothalamus were reported to be without effect on heroin self-administration [3]. Therefore, it is at present not clear whether the hypothalamus is involved in opiate reinforcement.

To address this issue, we have examined the effect of microinjections of the quaternary opiate antagonist methyl naltrexone in the hypothalamus on low-dose heroin self-administration. Methyl naltrexone was chosen because evidence suggests that of the various quaternary antagonists, it offers the optimum reduction in lipophilicity and retention of receptor binding affinity [5]. As a control in these experiments the efficacy of the same treatments were compared in the medial prefrontal cortex, a site which has been shown to be involved in psychomotor stimulant reinforcement [9]. We report here that quaternary antagonist pretreatments within the hypothalamus produce dose-dependent increases in in-

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

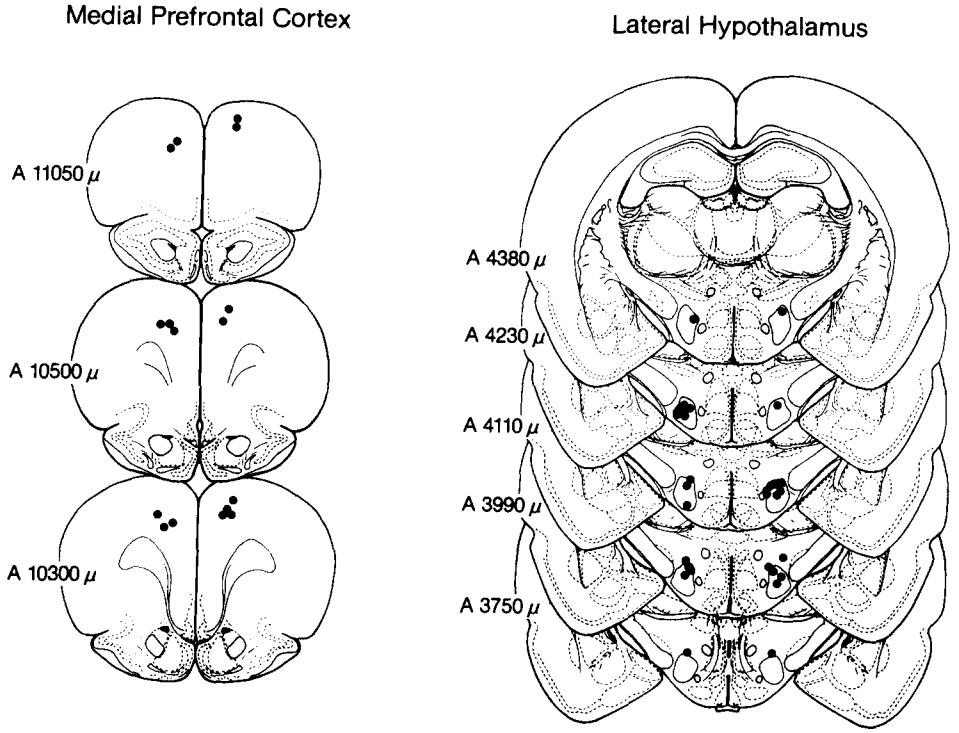

FIG. 1. Location of cannula tips in the medial prefrontal cortex and lateral hypothalamus. Schematic brain sections have been adapted from Konig and Klippel [11].

travenous heroin self-administration, whereas antagonist pretreatments within the medial prefrontal cortex are virtually without effect.

METHOD

Subjects 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.

Animals were first "shaped" to press a lever on a continuous reinforcement (CRF) schedule for food reinforcement while maintained at approximately 85% of their free-feeding weight by restricted feeding. This was done to facilitate acquisition of heroin self-administration once intravenous catheters were in place. Once trained to respond for food the animals were returned to ad lib feeding for the duration of the experiment. Several days later, each animal was surgically prepared under pentobarbital anesthesia (60 mg/kg, IP) with a chronic intravenous catheter constructed and implanted essentially as described by Weeks [17]. Animals were allowed to recover for 3–7 days.

Heroin self-administration sessions were carried out in dual-lever operant chambers, with responses on one lever leading to drug delivery under a CRF schedule (0.03 mg/kg/infusion; 100 microliters/kg; one-second drug infusion time), while responses on the other lever had no consequences for the subject but were recorded as a measure of lever-pressing behavior not paired with drug delivery. Drug delivery was followed by a signalled 5-second time-out period during which responding was again recorded but not reinforced; this period allowed the drug-delivery system time to reset. Drug was available during a single 90-min session each weekday, with weekends drug-free for all subjects.

Following acquisition of heroin self-administration, animals were implanted with bilateral guide cannulae directed towards the lateral hypothalamus (coordinates: −3 mm posterior to bregma; 3 mm lateral to midline; angled 10 degrees from the midline plane) or the medial prefrontal cortex (coordinates: +3 mm anterior to bregma; 1.9 mm lateral to midline; angled 20 degrees from the midline plane). Length of guide cannulae was such that they would not extend closer than 1 mm to the target site; injection cannulae which delivered the antagonist doses were of such length to reach the appropriate brain site.

At the conclusion of the experiment, animals were given an overdose of pentobarbital, and perfused transcardially with saline followed by 10% formalin. Brains were removed, and following further fixation and dehydration, were sliced into 50 μm sections which were mounted for histological determination of the cannula tips.

Brain antagonist treatments were given bilaterally 10 minutes prior to the start of the self-administration session; treatment days were separated by a minimum of 2 baseline or non-treatment days. The same dose range of methyl naltrexone as employed previously was used here (Corrigall and Vaccarino, in review), namely, 0 (saline vehicle), 0.1, 0.3, 1.0 and 3.0 micrograms total dose. This appears to be a dose range adequate to produce antagonism but below the threshold for non-specific and/or convulsive effects (e.g., see review in Brown and Goldberg). The antagonist was administered in a volume of 0.5 microliter per injection site and was delivered slowly over approximately 2 minutes by means of two micro-syringes driven by manually operated

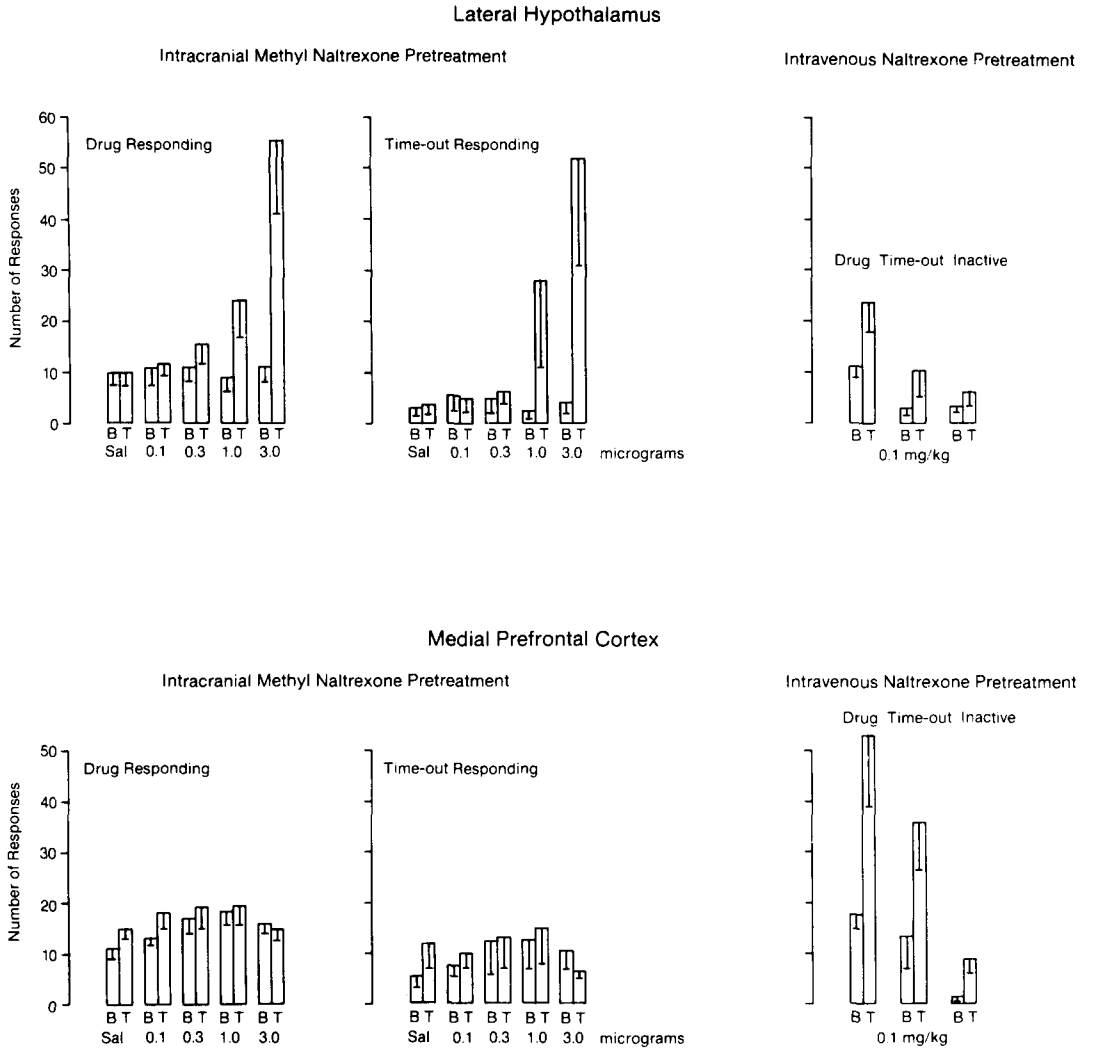

FIG. 2. Left and middle panels show values for DRUG and TIME-OUT components of responding on the drug lever during baseline sessions (B) and sessions in which animals received intracranial pretreatment with methyl naltrexone (T) in lateral hypothalamic and medial prefrontal cortical groups. Treatment doses shown below the bar graphs are in micrograms. Sample sizes are as follows: for lateral hypothalamic group, n=15; for the medial prefrontal cortex, n=8. Right hand panels show DRUG, TIME-OUT and INACTIVE responses during baseline sessions (B) and sessions in which animals received treatment with intravenous naltrexone (T) at the dose shown below the graphs. Sample sizes are as follows: for lateral hypothalamus, n=14; for the medial prefrontal cortex, n=8. In all cases, data are mean values; error bars show ±1 standard error of the mean (SEM).

micrometer screws which were advanced alternately in small increments so as to complete the bilateral injections over approximately the same 2-minute period. The volume of antagonist delivered was assessed by watching the advancement of a small bubble in a calibrated polyethylene line between the microsyringe and the rat. Since the animals were well habituated to handling, it was not necessary to forcibly restrain them during the microinjection procedure; they were merely held gently on the experimenter's lap.

In addition to the series of brain antagonist treatments, hypothalamic animals received two separate treatments with naltrexone (0.1 mg/kg IV) to provide comparisons for intracranial treatments. One of these naltrexone treatments was carried out before the series of intracranial methylnaltrexone injections, while the second was carried out after the intracranial series. Finally, after the series of brain microinjections and the second intravenous naltrexone treatment had been completed, responding of a subgroup of five of the lateral hypothalamic animals was examined during manipulation of the heroin dose available for self-administration. This was done to examine whether the effects of dose manipulation on responding were similar to those of antagonist treatment. The five subjects used in this experiment were animals which had not received the full series of brain injections of antagonist and were therefore excluded from the statistical analysis (see below); animals which had completed all treatments were not included in this dose manipulation since it was critical to process brains for histology before attrition due to physical loss of brain cannulae could occur.

Data reported for brain microinjections derives from animals completing all treatments and having satisfactory cannulae placements; this consists of 15 animals with can-

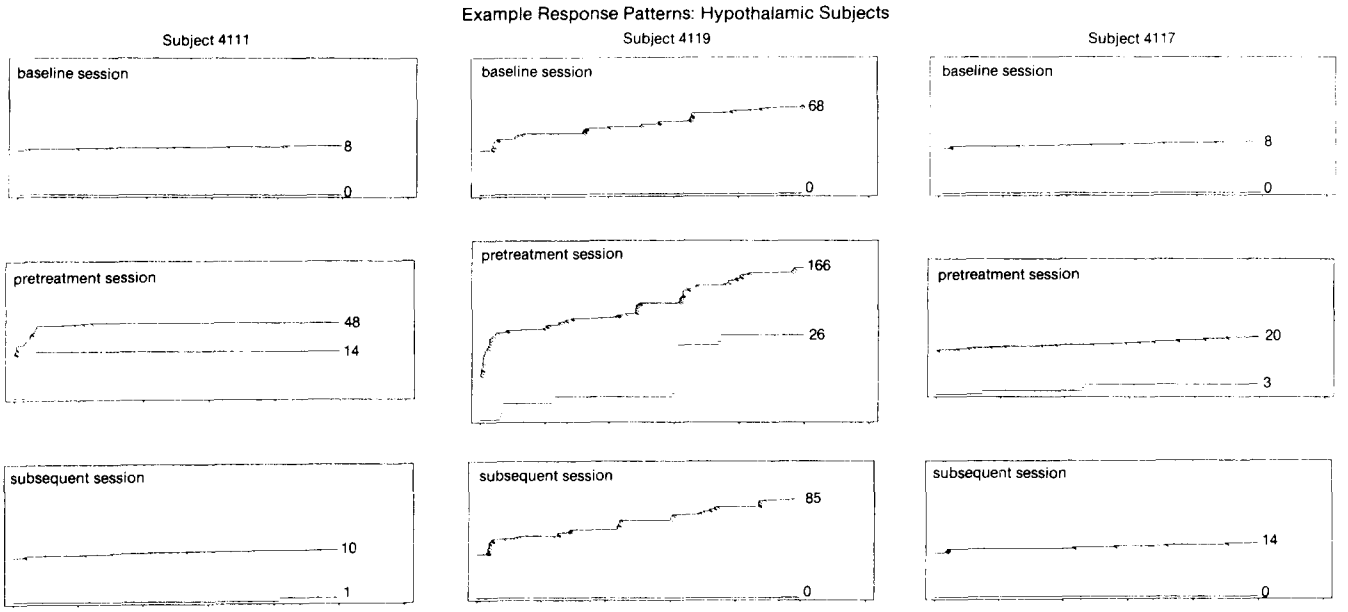

FIG. 3. Example cumulative records from three animals after treatment with 1.0 micrograms of methyl naltrexone, and during the session preceding and following (by one day) this treatment. In each panel the upper tracing is the record for the drug-reinforced lever, the lower is the record for the inactive lever. Numbers at the end of each tracing indicate the total responses on the respective lever during the session. Ticks in the downward-right direction on the record for the drug-reinforced lever indicate drug delivery. The cumulative records for the inactive lever have been expanded compared to those for the drug-reinforced lever for clarity; each step indicates a single inactive lever response. Cumulative records show responding for entire 90 min sessions; time marks below each pair of records indicate 18 min blocks.

nulae in the lateral hypothalamus and 8 with medial prefrontal cortex cannulae. For treatment with venous naltrexone, sample sizes are n=14 for the lateral hypothalamic group and n=8 for the medial prefrontal cortex. Analyses were performed on the following response components:

(1) responding on the drug paired lever during normal access periods of the sessions, i.e., when the time-out was not in effect (referred to as DRUG in the following discussion since such responding leads to drug delivery);

(2) responding during the signalled time-out periods of the sessions (denoted as TIME-OUT); and

(3) responding on the inactive lever (denoted as INACTIVE).

Statistical treatment was by analysis of variance of the difference between baseline and treatment session values after logarithmic transformation. This tranformation produces values which are more likely to be normally distributed, and is a valid transformation to use when data are of the nature of counts, since these may be skewed towards high values. The analysis of variance employed here was a mixed design in which antagonist treatment was examined within groups while effect on brain site was examined between groups.

RESULTS

Location of the tips of the injection cannulae are shown in Fig. 1. For the lateral hypothalamus the cannulae are all located within the same posterior lateral region examined in other studies of the role of this area in opiate reinforcement [3, 12, 13].

Effects of pre-session treatment with methyl naltrexone on responding on the drug-reinforced lever are shown in Fig. 2 for both lateral hypothalamic and medial prefrontal cortex groups. With respect to responding during normal access periods (i.e., the DRUG measure), inspection of the data shows that antagonist treatments within the lateral hypothalamus produced substantial dose-dependent increases in heroin-reinforced responding, whereas the same treatments in the medial prefrontal cortex had minimal effects upon the overall responding for heroin during the session.

These conclusions are generally supported by analysis of variance. For the DRUG response there was a significant site × dose interaction, DRUG: $F(4,84)=8.90$, $p<0.001$. To provide greater information about the changes over doses, the data were analyzed by trend analysis [7]. This means that the data were analyzed in terms of components (e.g., linear, quadratic, etc.) which describe the trend in the difference between baseline and treatment values. Tests of simple main effects showed a significant effect of dose of quaternary antagonist on the DRUG response for the hypothalamic group in both linear, $F(1,14)=16.27$, $p=0.001$, and quadratic, $F(1,14)=6.79$, $p<0.05$, trends; again these trends are visually evident in the data shown in Fig. 2. For the medial prefrontal cortex there was a significant effect of dose on the linear trend of the DRUG measure, $F(1,7)=33.6$, $p=0.001$. Although this trend is less evident than those in the hypothalamus, it becomes more understandable if one considers that the analysis is the difference between baseline and treatment measures, and notes that there is a generally consistent decreases in the difference between baseline and treatment measures with increasing dose of antagonist. In other words, in contrast to the prominent increases in antagonist effect in the hypothalamus with increasing dose, there is a small, linear decrease in treatment effect with dose in the medial prefrontal cortex.

The above effects are generally mirrored in responding during time-out periods. Statistical analysis showed a signifi-

TABLE 1

BASELINE AND INTRACRANIAL ANTAGONIST TREATMENT SCORES FOR RESPONDING ON THE INACTIVE LEVER (MEAN ± 1 STANDARD ERROR OF THE MEAN)

Antagonist Dose (micrograms)	Lateral Hypothalamus (n=15)		Medial Prefrontal Cortex (n=8)	
	Baseline	Treatment	Baseline	Treatment
0 (saline)	8.3 ± 6.8	8.3 ± 5.1	3.5 ± 2.0	2.9 ± 1.3
0.1	1.2 ± 0.7	8.7 ± 6.3	5.4 ± 3.8	1.4 ± 0.4
0.3	9.7 ± 6.6	4.3 ± 2.6	7.8 ± 4.8	3.1 ± 2.6
1.0	1.4 ± 1.1	104.7 ± 96.4	3.3 ± 1.5	2.3 ± 1.6
3.0	3.1 ± 1.3	60.9 ± 33.1	6.1 ± 4.6	4.1 ± 2.3

cant site × dose interaction in the TIME-OUT measure, $F(4,84)=6.18, p<0.001$. Tests of simple main effects showed no dose effect for the medial prefrontal cortex, $F(4,28)=1.40$, $p=0.260$, whereas for the lateral hypothalamus both linear, $F(1,14)=15.7$, $p=0.001$, and quadratic, $F(1,14)=4.78, p<0.05$, trends were significant.

Effects of brain antagonist treatments on inactive lever responding, which were generally not statistically significant, are shown in Table 1. With respect to inactive lever responding, there was no site × dose interaction, $F(4,84)=1.55$, $p=0.19$, and no effect of dose, $F(4,84)=1.76$, $p=0.14$, but there was an effect of brain site, $F(1,21)=16.1$, $p=0.001$, due to the effects in the hypothalamus.

In addition to increased responding for heroin after methyl naltrexone treatment, most animals displayed chewing and mouth grooming behaviors after treatment at the 3.0 microgram dose; three animals showed motor effects including locomotor stimulation and rearing. At the dose of 1.0 microgram, animals appeared to show more cage-chewing behavior than usual prior to the start of the self-administration session. Behavioral effects such as these were not observed after medial prefrontal cortex treatments.

Figure 3 shows examples of response patterns for three different hypothalamic subjects after pretreatment with 1 microgram methyl naltrexone, as well as during the preceding and following days' sessions. In the case of subject 4111, antagonist treatment caused a marked increase in responding on the drug lever beginning immediately at the start of the session. A bout of responding on the inactive lever commenced in the middle of the episode of drug-lever responding. Two features of the responding on the drug lever are of note, one being the large increase in drug-lever responding at the beginning of the session, the other being the complete cessation of responding for approximately the last three-quarters of the session. Subject 4119, an animal which responded at high rates in the non-treatment sessions, responded substantially on the drug lever at the beginning of the session before emitting responses on the inactive lever, but continued to respond on the drug lever consistently, and on the inactive lever intermittently, throughout the session. Considering another example (subject 4117), antagonist pretreatment produced a sustained increase in drug lever responding throughout the heroin session in the virtual absence of inactive lever responding. Response patterns such as these illustrate that increased activity on the drug-reinforced lever can occur in isolation from increases on the inactive lever, but that when the two occur together the choice of the inactive lever typically occurs temporally after responding on the active level.

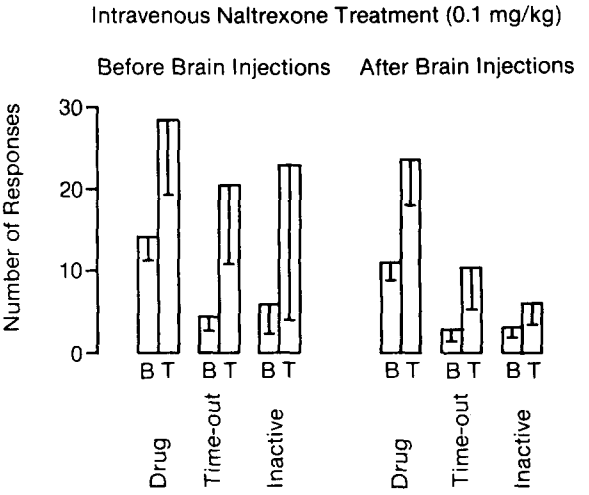

FIG. 4. Comparison of the effects of pretreatment with intravenous naltrexone in the hypothalamic group both before and after the series of brain antagonist treatments had been done. Data are mean values (±1 SEM); n=14.

Changes in responding produced by systemic treatment with antagonist were examined in both sets of animals. Results of pre-session treatment with naltrexone, carried out following the series of brain injections, are shown in Fig. 2. Analysis of variance with one between subject factor (brain site) and one within subject factor (naltrexone treatment) showed that there was a significant naltrexone effect on each of the DRUG, $F(1,21)=14.3$, $p<0.05$, TIME-OUT, $F(1,21)=16.8$, $p<0.05$, and INACTIVE, $F(1,21)=5.41$, $p<0.05$, measures, but with no effect of brain site on any measure [DRUG, $F(1,21)=1.18$, $p=0.29$; TIME-OUT, $F(1,21)=0.90$, $p=0.35$; INACTIVE, $F(1,21)=2.76$, $p=0.11$].

Comparison of the effects of pre-treatment with intravenous naltrexone both immediately before and after the sequence of brain infusions of methyl naltrexone had been completed are shown in Fig. 4 for the hypothalamic subjects. The response increase on the drug-reinforced lever during periods of availability was similar in both treatments, but there was a smaller increase in both time-out and inactive lever responding in the second treatment.

As a qualitative assessment of the effects of manipulation of the heroin dose available to the subjects, animals which had acquired self-administration at 0.03 mg/kg/infusion were tested at heroin doses of 0.01 and 0.001 mg/kg/infusion (Fig. 5). There were compensatory response increases on the drug-reinforced lever (both DRUG and TIME-OUT measures) when the unit dose of heroin was decreased from 0.03 to 0.01 mg/kg and also from 0.01 to 0.001 mg/kg, but responding on the inactive lever did not increase until the unit dose was changed to 0.001 mg/kg, a dose which failed to maintain consistent behavior, and a dose at which animals' drug intake was markedly reduced.

DISCUSSION

These experiments show that microinjections of the quaternary antagonist methyl naltrexone into the lateral

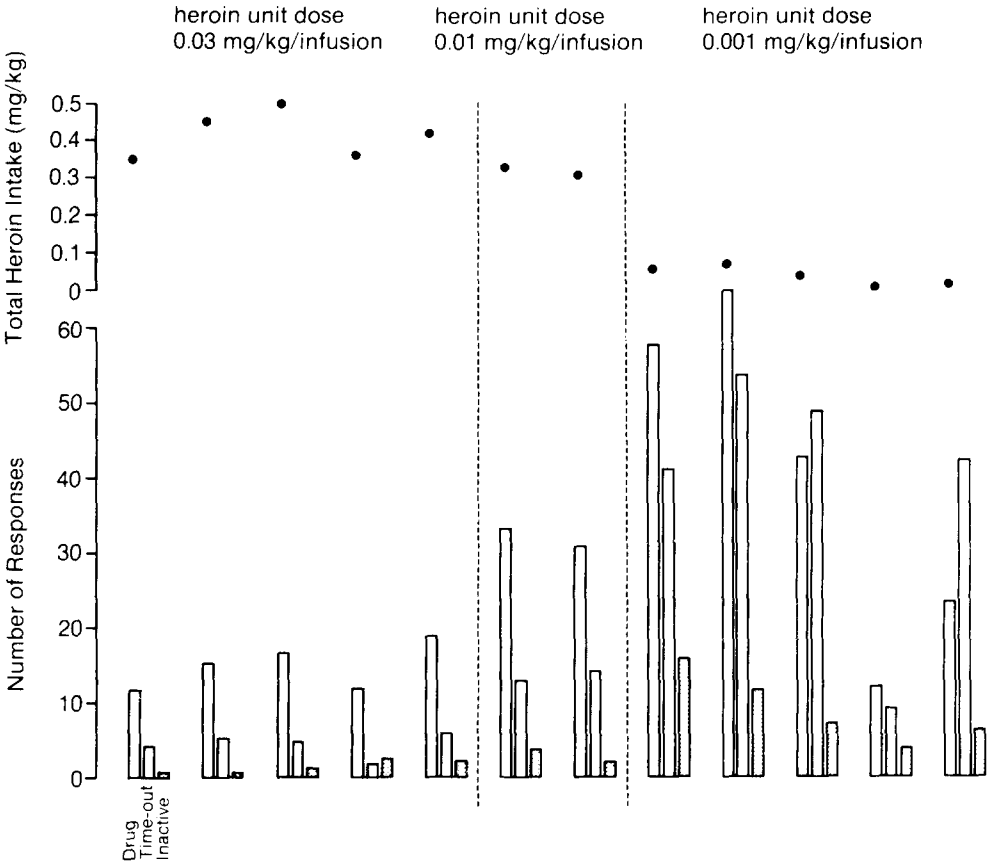

FIG. 5. Effect of reducing the dose of heroin available for self-administration on response patterns and average heroin intake; n=5. Bar graphs show DRUG, TIME-OUT and INACTIVE measures during successive sessions; vertical lines indicate changes in unit dose of heroin available to the animals. The upper graph shows the total heroin intake in each session. Data are mean values.

hypothalamus of animals trained for intravenous heroin self-administration produce significant dose-dependent increases in responding on the drug manipulandum. The increases are similar to those observed when animals receive an intravenous challenge with naltrexone, or when the dosage of available heroin is reduced. In contrast, microinjections of the same doses of antagonist into the medial prefrontal cortex did not lead to any significant increases in responding. Indeed, the only significant effect of injections into the medial prefrontal cortex was a small decrease in difference between baseline and treatment sessions with increasing antagonist dose which is unlikely to be of biological significance. The lateral hypothalamic sites are therefore clearly involved in regulating the intravenous intake of heroin in a manner such that reduction of the effective local dosage leads to increased drug-taking.

These findings are consistent with Olds' observations [12,13] that animals will respond for injections of opiate agonists into the same area of the hypothalamus which was examined here. However, the present findings conflict with the study carried out by Britt and Wise [3] which showed that kainic acid lesions in this hypothalamic region did not significantly alter self-administration of either heroin or cocaine. It is difficult to specify reasons for this discrepancy. The lesion study used a regimen of alternating daily access to heroin and cocaine in which alternate-day access to cocaine reinforcement might tend to sustain heroin responding at higher levels than otherwise would occur. However, this factor alone is unlikely to account for the total lack of effect observed after kainic acid treatment. Another difference is that the animals in the lesion study were tested after a six-day absence from self-administration, which may make the data more comparable to re-acquisition than to maintenance. In addition, the effect of the lesions may be transient. For example, some electrolytic lesions have been reported to have only short-duration effects on morphine self-administration [8]; possibly that part of the regulation of heroin self-administration normally supported by hypothalamic neurons is rapidly assumed by other brain sites, tending therefore to mask deficits which might otherwise be observed. While such possibilities are purely speculative, they do point out the need to utilize rapidly acting, short-duration probes in addressing the issue of brain substrates regulating drug self-administration.

Although there were no significant effects of antagonist treatment in the lateral hypothalamus on inactive lever responding, there were variable increases produced as a result of different subject-to-subject effects, representative samples of which are shown in Fig. 3. Similar but more reliable increases were seen following treatment with naltrexone systemically. These would be consistent with a more widespread effect on brain systems regulating drug intake as

compared to the localized effect achieved with hypothalamic antagonism. Similar increases occurred during reduction of the available heroin dose, but only when the unit dose was so low that it was difficult for animals to maintain non-zero levels of drug intake. It would appear, therefore, that responding on the inactive lever occurs to some degree when the efficacy of self-administered drug is reduced either with an antagonist or by actually decreasing the available dosage. It is interesting that Olds [12] also reported inactive lever response increases during extinction trials as well as during trials in which morphine and naloxone were delivered together into the hypothalamus.

It is possible that the effects of antagonist treatment in the lateral hypothalamus on heroin-reinforced responding could have their basis in physical dependence. That is, antagonist treatment within the hypothalamus might precipitate withdrawal symptoms which the animals seek to overcome by taking increased numbers of infusions of agonist. Indeed, some behavioral effects which could be interpreted as withdrawal-like were observed after treatment with the highest doses of antagonist. On the other hand, it is unlikely that physical dependence would develop in animals in which daily drug intake is so low (approximately 10–15 infusions of 0.03 mg/kg, yielding 0.30 to 0.45 mg/kg/day) and with a schedule of access in which weekends were drug-free. Furthermore, baseline intake of heroin across successive weeks was very stable, and did not show any upward trend suggestive of development of tolerance or physical dependence. In addition, the two treatments with intravenous naltrexone, one before and one after the series of brain injections, were separated by a period of one month of heroin self-administration. Consequently, if the subjects were becoming increasingly physically dependent during self-administration, their responding should be more affected by the second as compared to the first treatment with intravenous naltrexone, whereas in fact the opposite result was observed. The reduction in naltrexone effect on the second test may be due to the animals' greater familiarity with the operant chambers and treatment effects.

With respect to the medial prefrontal cortex, there is no evidence from this research to suggest that this brain area participates in regulation of intravenous self-administration. While this structure has not previously been investigated as a substrate of opiate self-administration, it has been shown to support intracranial cocaine self-administration [9]. Therefore the present study provides further evidence for a dissociation of substrates mediating opiate and psychomotor stimulant reinforcement.

The fact that one can demonstrate an ineffective brain site is important as a control even though frontal cortical areas have at best moderate opiate receptor densities while the hypothalamus contains high densities of receptors [1]. In spite of these differences in receptor densities, the lack of effect following medial prefrontal cortex treatment indicates that brain injections into receptor-containing areas are not sufficient to produce effects on intravenous self-administration. This claim, however, must be tempered with the proviso that the receptor subtypes in the prefrontal cortex may be different from those in the hypothalamus, and if so might require higher doses of antagonist to produce observable effects. Unfortunately there is no information available at present on the densities of receptor subtypes in these two brain regions. Nevertheless the lack of effect in the medial prefrontal cortex is valuable information in view of the fact that quaternary antagonists have been shown to be effective in modifying heroin self-administration when injected into several sites, specifically, the cerebral ventricles [16], the ventral tegmental area [4,15], the nucleus accumbens ([15], Corrigall and Vaccarino, in review), the periaqueductal grey (Corrigall and Vaccarino, in review), as well as the hypothalamic sites reported here.

In summary, the data reported here show that localized opiate antagonist treatment within the posterior lateral hypothalamus causes increases in responding for intravenous heroin similar to those which occur after systemic antagonist treatment or reduction of the available heroin dose. The data support the hypothesis that these hypothalamic sites are involved in the regulation of heroin self-administration, and may comprise one of the neural substrates of opiate reinforcement.

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