Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 491–498, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00015-1

Effects of Restraint Stress and Intra-Ventral Tegmental Area Injections of Morphine and Methyl Naltrexone on the Discriminative Stimulus Effects of Heroin in the Rat

YAVIN SHAHAM[1] AND JANE STEWART

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 1455 de Maisonneuve Blvd., Montreal, Quebec, Canada, H3G 1M8

Received 20 April 1994

SHAHAM, Y. AND J. STEWART. *Effects of restraint stress and intra-ventral tegmental area injections of morphine and methyl naltrexone on the discriminative stimulus effects of heroin in the rat.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 491–498, 1995.—The effect of restraint stress on the discriminative stimulus properties of heroin and the role of the opioid receptor activation in the ventral tegmental area in heroin discrimination were examined. In Experiment 1, male rats were trained to discriminate heroin (0.5 mg/kg, SC) from saline under conditions of exposure to restraint (15 min/day; three times a week) or no stress. Dose-response curves were subsequently determined under conditions of no stress, restraint, corticosterone (3 mg/kg, IP), and saline. Exposure to restraint during training did not alter heroin discrimination under any of the conditions tested. In contrast, administration of restraint or the stress hormone corticosterone just prior to drug injections decreased sensitivity to the heroin cue. In Experiment 2, injections of morphine (5–10 μg/side) into the ventral tegmental area (VTA, the cell body region of the mesocorticolimbic dopamine neurons) did not result in heroin-appropriate responding in animals trained to discriminate heroin injected systemically from saline. Furthermore, intra-VTA injections of the opioid antagonist methyl naltrexone (0.75–3.0 μg/side) did not block the discriminability of heroin given systemically. These results indicate that exposure to restraint stress or the stress hormone corticosterone in close temporal contiguity to the drug injection may reduce the sensitivity to the opioid cue. In addition, under the condition of the present experiment activation of opioid receptors in the VTA does not appear to mimic the discriminative stimulus effects of systemically administered heroin.

Drug discrimination Heroin Methyl naltrexone Morphine Mesocorticolimbic dopamine system Opioids Stress Ventral tegmental area

RECENTLY we have shown that in the rat repeated exposure to repeated mild restraint or foot shock immediately before drug sessions increases the self-administration of opioids, suggesting that certain stressors enhance the reinforcing properties of opioids under these conditions (30,31,33). Stressors might contribute to this enhancement by changing the sensitivity of the animal to the presence of the drug in the body. To test this idea, we studied in rats the effects of restraint stress on the discriminative stimulus properties of heroin measured by the drug discrimination method [see (4) for a review]. These effects of opioids are thought to be related to the reinforcing properties of opioids in animals and to their abuse liability and subjective effects in humans (5). One reason for thinking that exposure to stress might alter the sensitivity of rats to the opioid cue is that chronic deprivation conditions, such as food or social deprivation, have been reported to increase sensitivity to the discriminative stimulus effects of stimulant or opioid drugs [(12,13); but see (40)].

[1] Requests for reprints should be addressed to Dr. Yavin Shaham, Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Room 1013, Concordia University, 1455 de Maisonneuve Blvd., Montreal, Quebec H3G 1M8, Canada.

Another issue explored in the present study was the neurochemical site of action of heroin underlying its discriminative stimulus properties. If, for example, the neural systems underlying the discriminative properties of heroin were the same or similar to those underlying its reinforcing properties, the mesocorticolimbic dopamine (DA) system might be expected to be involved. This brain system consists of cell bodies that lie mainly in the ventral tegmental area (VTA) of the mesencephalon and project to limbic (e.g., nucleus accumbens) and frontal cortical areas (1). The mesocorticolimbic DA system has been implicated in the behavioral activating and reinforcing effects of opioid and stimulant drugs [see (20,43)].

Previous research indicates that the discriminative stimulus effects of opioids are centrally mediated (24,41), but opioid agonists such as heroin and morphine have, of course, many actions in the CNS, any one or combination of which could potentially serve as the basis for the discrimination of its presence. Although several studies have been done to determine the brain site(s) involved in the stimulus properties of opioids, no consistent findings have emerged. Krynock and Rosecrans (22), for example, reported that intra-periaqueductal grey (PAG) injections of morphine (0.5–4.0 μg) mimic the opioid cue in rats trained to discriminate morphine injected systemically from saline. This effect, however, was not observed by Shannon and Holtzman (35), who used similar training conditions. Recently, it was reported that intra-VTA or intra-accumbens injections of low doses of morphine (1–3 μg) resulted in full or partial substitution, respectively, for morphine in rats trained to discriminate morphine (3 mg/kg, SC) from saline (38). In contrast, Jaeger and Van Der Kooy (17,18) have reported that injections of morphine into the parabrachial nucleus, but not into the VTA or the nucleus accumbens, substitute for the morphine cue in the conditioned taste aversion model for drug discrimination. Van Ree et al. (42) reported that injections of low doses of fentanyl or β-endorphin into the nucleus raphe magnus result in drug-appropriate responding in animals trained to discriminate fentanyl (0.04 mg/kg, SC) from saline. Importantly, however, electrolytic lesions of the raphe nuclei do not block the cue properties of morphine injected systemically (29).

Efforts to specify anatomical sites of action of intracranially administered drugs have seldom been done in these studies. These include injecting the drugs to sites dorsal to the site of interest (which would serve as a control for the spread of drugs up the outside of the cannula shaft) and attempts to avoid penetration of the ventricles in close proximity to the injections regions (which would prevent the rapid spread of drugs via the ventricles) [see (44) for a discussion of these problems]. It is not possible, therefore, to determine which set of results is the most reliable. In addition, no attempt was made in the studies reviewed to block the stimulus cue of opioid agonists injected systemically by administration of hydrophilic opioid antagonists into the brain sites examined. This would seem to be important in light of the finding by Locke and Holtzman (24) that intracerebroventricular (ICV) administration of naltrexone methylbromide blocks the stimulus control of behavior based on systemically administered morphine.

In the present experiments, we first examined whether exposure to restraint stress would alter the discriminative stimulus effects of heroin injected systemically (Experiment 1). This was explored initially by examining the effect of previous exposure to restraint stress on the dose-response curve for heroin discrimination. In addition, we examined the effect on heroin discrimination of both restraint stress and an injection of corticosterone administered just before the drug injection during tests for dose–response determination. We chose to examine the effect of repeated exposure to short-term restraint stress on heroin discrimination because of our previous studies demonstrating that this stressor increases oral opioid self-administration (30,31). Corticosterone injections were used because levels of this hormone increase under stress, and it has been implicated in the initiation of self-administration of drugs of abuse [e.g., amphetamine, see (28)]. In Experiment 2, we examined whether activation of the mesocorticolimbic DA system by injections of morphine into the cell body region of the mesocorticolimbic DA system in the VTA [see (11,37)] would result in heroin-appropriate responding in animals previously trained to discriminate heroin, injected systemically, from saline. In this experiment we also determined whether intra-VTA injections of the opioid antagonist methyl naltrexone would block the stimulus effects of systemically injected heroin.

METHOD

Subjects

Sixteen male Wistar rats (Charles River) weighing 250–300 g at the start of the experiment were housed individually in stainless steel cages with tap water continuously available. Access to food was restricted to 18–20 g of standard rat chow provided once each day. Testing was conducted during the dark phase of a 12L : 12D cycle.

Drugs

Heroin (diacetylmorphine HCl, Health and Welfare Canada, Ottawa) was dissolved in physiological saline and injected SC. Morphine was injected intracranially at doses of 0.0, 5.0, and 10.0 μg/side. Morphine was used for the intracranial injections rather than heroin because when given systemically heroin is rapidly hydrolyzed to 6-monoacetylmorphine, which, in turn, is hydrolyzed to morphine. These metabolites, rather than heroin itself, are responsible for the pharmacological actions of heroin injected systemically (19). Methyl naltrexone was injected intracranially at doses of 0.0, 0.75, 1.5, and 3.0 μg/side. This opioid antagonist, a quaternary analogue of naltrexone, was chosen because it has low lipophilicity while retaining potency at the opioid receptor, conditions that would help to localize the drug to the injection site (3). Corticosterone-21-hemisuccinate (3 mg/kg, dose expressed as a base) was dissolved in physiological saline and injected IP. This dose of corticosterone has been shown to alter *d*-amphetamine self-administration in rats (28).

Surgery and Histology

Animals in Experiment 2 were anesthetized with sodium pentobarbital (65 mg/kg, IP) and implanted bilaterally into the VTA with 22-ga stainless steel guide cannulae (Plastic One, Inc., Roanoke, VA). With the stereotaxic arms angled at 16° from the vertical plane, and the incisor bar 5.0 mm above the interaural line, cannulae were aimed 1.0 mm above the VTA using the skull surface coordinates of 3.6 mm posterior to bregma, 3.6 mm lateral to the midline, and 8.1 mm ventral from the skull surface. The cannulae were anchored to the skull with jeweler's screws and dental cement, and stainless steel obturators were inserted such that the tips extended 1 mm below the end of the cannulae. At the end of the experiment, rats were overdosed with chloral hydrate and perfused transcardially with 0.9% saline followed by 10% formalin.

The brains were removed, sliced in 30-μm frozen sections, and stained with formal thionin.

Apparatus

The experiments were conducted in eight operant chambers (19 × 23 × 29 cm or 19 × 31 × 21 cm), each constructed of two aluminum walls, two Plexiglas walls, a Plexiglas ceiling, and a stainless steel rod floor. Each chamber contained two levers stationed 5 or 11 cm apart on one of the aluminum walls, a food hopper (a 4.5 × 4.5 × 4.5 cm alcove) positioned midway between the levers, and a house light situated on the ceiling.

Procedure

In Experiment 1, the animals were divided into two groups. Animals in the stress group (n = 8) were exposed to restraint (restrainers were made of a 25 × 6 cm Plexiglas base with a foam-padded wire mesh cover) for 15 min, three times a week, 1 h prior to the drug/saline injections. This regimen of exposure to restraint stress was used because it has been shown in our previous work (32) to enhance the effect of morphine on locomotor activity in rats. Exposure to restraint was equally distributed between the saline and the heroin training sessions that were conducted once per day for 5–7 days/week. Animals from the control group (n = 8) were not exposed to restraint during the training sessions. Each session was preceded by a SC injection of either 0.5 mg/kg of heroin or 1 ml/kg of saline, 20 min before placement in the operant chamber. Following a 2-min adaptation period in the operant chambers, the house light was turned on and the rats could obtain food by responding on either the heroin lever or the saline lever. The heroin and saline trials were presented randomly, but neither solution was administered on more than two consecutive sessions. Rats were initially trained to lever press for food (45-mg pellets; P. J. Noyes Company Inc., Lancaster, NH) during 30-min sessions. The schedule requirements were increased over a 10-day period from fixed-ratio-1 (FR-1, each lever press is reinforced) to FR-16. Subsequent sessions were terminated either after 15 min or after the animals obtained 50 pellets. Responses on the "incorrect" lever had no consequences. Training continued until at least 80% of the responses emitted prior to the first reinforcement were on the appropriate lever on 8 out of 10 consecutive sessions. Subsequently, dose-response determinations for heroin discrimination were determined under four experimental conditions: no stress, exposure to restraint, injection of corticosterone, or injection of saline 15 min prior to injection of heroin or saline. Each of the four dose-response determinations was conducted over a 2-week period during which retraining sessions with saline or 0.5 mg/kg heroin were given between the tests for generalization to heroin at the doses of 0.0625, 0.125, and 0.25 mg/kg. During each 2-week period when the dose-response determinations were being made under one of the four conditions, the retraining sessions with saline and 0.5 mg/kg heroin were also conducted under the same conditions. During the generalization tests, the lever providing reinforcement was the lever selected by the rat initially. The selected lever was that lever on which 16 responses occurred. Responses were reinforced for the first and each subsequent completion of the FR-16 requirement on this lever. Responses on the alternative (nonselected) lever had no programmed consequences.

Experiment 2 started 1 month after the completion of Experiment 1. Thirteen rats from Experiment 1 were implanted with bilateral guide cannulae aimed at the VTA. Following a 2-week recovery period, rats were given discrimination training in the absence of stress until they all reached the discrimination criterion. Dose-response curves were determined under two conditions. In one, rats were tested following bilateral injections of morphine into the VTA (0.0, 5.0, and 10.0 μg/side) given 10–15 min prior to a SC injection of saline; in the other, rats were tested after bilateral injections of methyl naltrexone into the VTA (0.0, 0.75, 1.5, and 3.0 μg/side) given 10–15 min prior to a SC injection of 0.5 mg/kg of heroin. Under both the intra-VTA morphine and the methyl naltrexone conditions, rats were placed in the operant chambers 20 min after the SC injections; after the usual 2-min delay, the house light was illuminated, and the lever providing reinforcement was determined as described previously.

RESULTS

Experiment 1

Data from 13 of the 16 animals were used in the analyses; three animals did not meet the discrimination task criterion. The monotonic portions of the dose-response curve for heroin-appropriate responding were subjected to a logistic regression using the Nonlin procedure (Systat Inc., Evanston, IL). Table 1 presents ED_{50} values and 95% confidence intervals for the experimental groups. Control and stress groups had similar ED_{50} values within each of the four experimental conditions, indicating that exposure to restraint during training did not alter the sensitivity for the discriminative stimulus effects of heroin under any of the test conditions. No significant group differences were observed for response rate (data not shown).

TABLE 1

ED_{50} VALUES (mg/kg OF HEROIN) AND 95% CONFIDENCE INTERVALS FOR HEROIN-APPROPRIATE RESPONDING DOSE-RESPONSE CURVES UNDER CONDITIONS OF NO STRESS, RESTRAINT, CORTICOSTERONE INJECTIONS, AND SALINE INJECTIONS

	Control Group		Stress Group		Combined Groups	
	ED_{50}	95% Confidence Interval	ED_{50}	95% Confidence Interval	ED_{50}	95% Confidence Interval
No stress	0.15	0.11–0.19	0.13	0.03–0.23	0.14	0.10–0.18
Restraint	0.23	0.18–0.28	0.19	0.11–0.27	0.21	0.17–0.25
Corticosterone (3 mg/kg)	0.22	0.17–0.27	0.20	0.15–0.25	0.21	0.18–0.24
Saline	0.16	0.09–0.23	0.13	0.09–0.17	0.15	0.11–0.18

On the other hand, although there were no differences between the control and stress training conditions, the ED_{50} values varied significantly as a function of the conditions of the dose-response determination. Regardless of the training condition, exposure to restraint or corticosterone just prior to the discrimination session caused a significant increase in ED_{50} values over those obtained under the no-stress or saline injection conditions. These results suggest that under conditions of restraint stress or exposure to corticosterone, animals were less sensitive to the heroin cue. The dose-response curves for percent of heroin-appropriate responding under the four conditions for the two groups combined are shown in Fig. 1a. Neither restraint nor corticosterone altered response rates (Fig. 1b). Table 2 shows the percent of heroin-appropriate responding for individual animals in the medium dose of heroin (0.125 mg/kg).

Experiment 2

All animals met the discrimination task criterion within 18 days. The data from 4 of the 13 animals were excluded because the cannulae were not properly placed. The locations of the cannulae tips of the remaining animals are presented in Fig. 2.

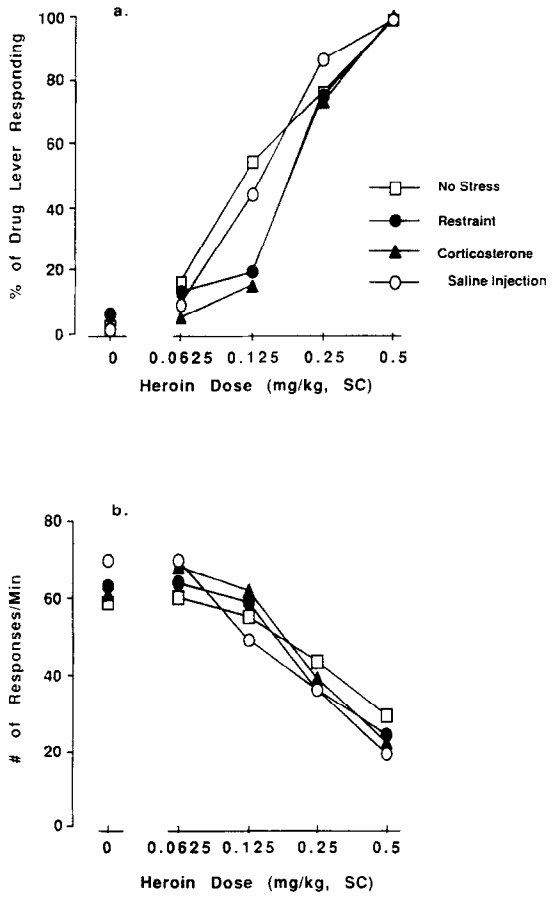

FIG. 1. Stimulus generalization tests for systemic heroin injections. (a) Mean percent heroin-appropriate responding under conditions of no stress, 15 min of restraint, corticosterone (3 mg/kg, IP) injection, and saline injection. (b) Mean response rates under these same conditions.

The stimulus generalization test for the highest dose of methyl naltrexone (3 μg/side) was conducted in only six animals. This high dose caused extreme irritability, rotational behavior, and head swaying. The dose-response curves and response rates obtained following intra-VTA morphine and SC injections of saline, and intra-VTA methyl naltrexone and SC injection of 0.5 mg/kg heroin, are presented in Figs. 3 and 4. Intra-VTA infusions of morphine did not result in heroin-appropriate responding (Fig. 3a); neither did intra-VTA infusions of methyl naltrexone block the discriminative stimulus effect of systemic heroin (Fig. 4a). Administration of morphine into the VTA, did, however, cause a dose-dependent decrease in response rate ($p < 0.05$). Administration of methyl naltrexone did not significantly alter rate of responding after systemic injection of heroin, with the exception of a large decrease in response rate at the highest dose.

DISCUSSION

Two main findings emerge from the present experiments. First, exposure to restraint or to the stress hormone, corticosterone, just prior to drug injection modestly decreased sensitivity to the heroin cue. Second, infusions of morphine into the VTA, where it is known to activate the mesocorticolimbic DA neurons (11,37), did not mimic the discriminative stimulus effects of systemically administered heroin. In addition, infusions of methyl naltrexone into this brain region did not block heroin discrimination. These findings are discussed in turn.

The observation that exposure to restraint just before the drug injection does not increase, and, in fact, somewhat decreases sensitivity to the heroin cue is partly unexpected in view of the results of Fowler et al. (12) and Gaiardi et al. (13). Although the conditions of stress exposure were very different, these authors report that social or food deprivation increases the discriminability of stimulant drugs or of morphine, respectively. An obvious explanation for the present results might be that exposure to restraint just before the discrimination sessions disrupts the performance of any learned behavioral response [cf. (15)]. This possibility seems unlikely in that neither exposure to restraint nor the corticosterone injections interfered with performance when either the training dose or saline was injected.

Alternatively, the differences between the results of the present experiment and those of others are more probably due to the types of stressor used and to their physiological consequences. Acute exposure to restraint is associated with reliable physiological changes (i.e., the stress response), including increased plasma levels of corticosterone, ACTH, endorphins, and catecholamines (23,25). In contrast, conditions of isolation (14,16) or food deprivation [(9); but see (2)] do not lead to consistent changes in these physiological measures. The fact that an injection of corticosterone mimics the effect of restraint stress lends support to this idea.

An additional finding in Experiment 1 was that, unlike acute exposure to restraint given just prior to tests to determine the dose-response for heroin discrimination, repeated exposure to restraint given three times per week during discrimination training did not alter the subsequent discriminability of heroin during testing for dose-response determinations. These results are similar to those of Miczek (26), who reported that exposure to a defeat stress during training of a morphine discrimination did not alter the sensitivity to a morphine cue.

In previous studies we have shown that restraint stress given just before the drug sessions increases oral morphine

TABLE 2

PERCENT OF HEROIN-APPROPRIATE RESPONDING FOR INDIVIDUAL ANIMALS AT THE DOSE OF 0.125 mg/kg HEROIN DURING THE TESTING PHASE

		Testing Conditions			
Training Conditions	Subject	No Stress	Saline Injection 15 min Prior to the Drug Injection	Corticosterone Injection 15 min Prior to the Drug Injection	Exposure to Restraint for 15 min Prior to the Drug Injection
No stress condition	1	84	70	100	16
	2	0	0	0	0
	3	100	100	0	0
	4	6	0	0	16
	5	55	0	0	0
	6	33	100	0	0
	7	33	0	0	0
Stress condition (Restraint, 15 min, 3 times weekly, 1 h prior to training sessions)	1	0	89	0	11
	2	100	100	100	100
	3	100	11	0	0
	4	100	100	0	100
	5	0	0	0	0
	6	89	0	0	0

and fentanyl self-administration (30,31) and enhances sensitization to the locomotor stimulating effects of morphine that are observed following repeated intermittent administration of this drug (32). Thus, it appears that exposure to restraint differentially alters the discriminative stimulus and the reinforcing or the behavioral activating effects of opioids. It may be argued, however, that this difference is related to differences in the temporal relation between exposure to stress and drugs in these studies. Restraint stress-induced enhancement of opioid reinforcement or locomotor activity was most pronounced when exposure to restraint stress was paired with the drug exposure. These effects also occurred, however, when

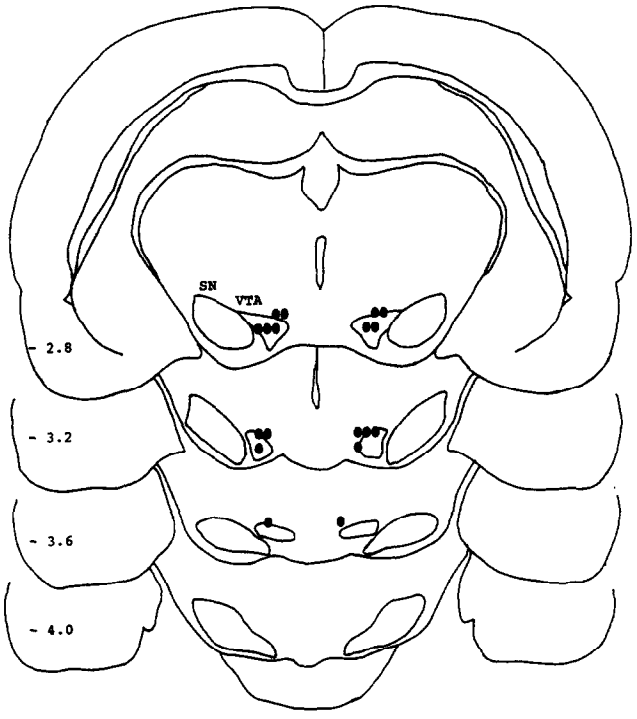

FIG. 2. Location of injector cannula tips. The numbers at each coronal section correspond to the distance from bregma (27).

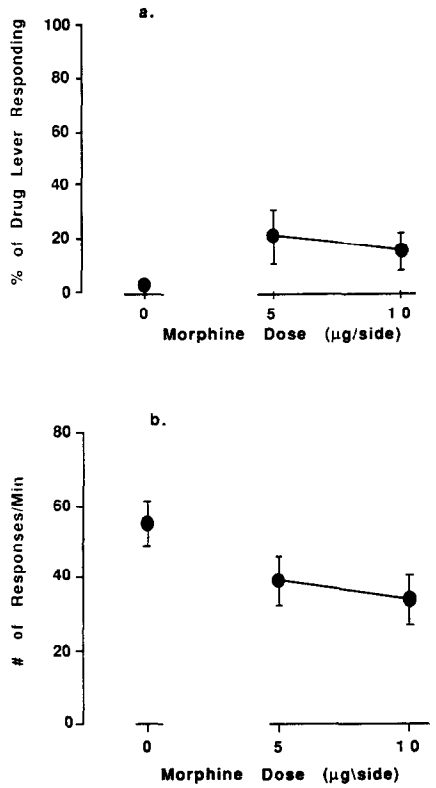

FIG. 3. Stimulus generalization tests for intra-VTA morphine injections. (a) Percent heroin-appropriate responding for intra-VTA injections of morphine followed by SC saline injections. (b) Mean response rates under these same conditions.

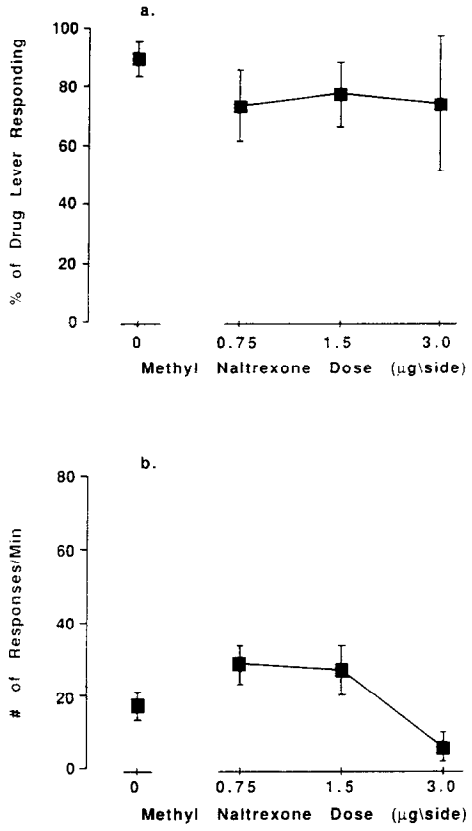

FIG. 4. Stimulus generalization tests for intra-VTA methyl naltrexone injections. (a) Percent heroin-appropriate responding for intra-VTA methyl naltrexone injections followed by heroin (0.5 mg/kg, SC) injections. (b) Mean response rates under these same conditions.

exposure to restraint was only partially paired with the drug consumption sessions [see (30)]. In contrast, when exposure to restraint was explicitly unpaired with the drug, no changes in opioid effects were observed in our studies. It should be noted, however, that in the training phase of the present experiment it was not possible to parallel exactly the procedure of pairing and unpairing restraint stress with drug used in our previous studies. It was feared that the restraint procedure, itself, would serve as a cue for responding in the discrimination task. On the other hand, during the dose-response determinations, animals were repeatedly exposed to restraint just before drug injections. The only effect of restraint under these conditions was to reduce the effectiveness of the heroin cue. Thus, although exposure to restraint was not explicitly paired with the drug injections in Experiment 1, it was partially paired, making it unlikely that temporal factors alone could account for the differential effect of restraint stress on the discriminative stimulus and the reinforcing or the behavioral activating effects of opioids.

One possible reason for the different effects of restraint stress on self-administration, behavioral activating effects, and discriminative stimulus effects of opioids is that these opioid effects are mediated by different neuronal systems. As mentioned before, the cell body region of the mesocorticolimbic DA system in the VTA is known to be an important site for the reinforcing and behavioral activating effects of opioid drugs. In the present experiment, intra-VTA injections of morphine, at doses that cause behavioral activation (32), that are self-administered (10), and that reinstate previously extinguished heroin-reinforced behavior (39), did not mimic the discriminative stimulus effects of heroin. In addition, intra-VTA injections of methyl naltrexone, at doses that block the stimulus cue of morphine when administered ICV (24), failed to alter the discriminative stimulus properties of heroin injected systemically. Thus, it appears that the mesocorticolimbic DA system does not play a major role in the discriminative stimulus effects of opioid agonists [also see (17,18)]. This conclusion is challenged, however, by the recent demonstration by Shoaib and Spanagel (38) that intra-VTA injections of morphine result in morphine-appropriate responding in animals trained to discriminate morphine from saline.

One factor that might account, in part, for the inconsistent findings is the training dose of the drugs used. The role of DA in opioid discrimination has been examined in several studies using different training doses of opioids. In studies using morphine (3.0–5.6 mg/kg), fentanyl (0.005–0.4 mg/kg), or heroin (0.3 mg/kg) as the training drugs, dopamine agonists (*d*-amphetamine or apomorphine) do not substitute for the opioid cue (6,7,21,34,45), nor do DA antagonists block opioid discrimination at a range of doses that do not severely impair response rate (6,8). In a study by Shannon and Holtzman (36), however, in which a relatively low training dose of morphine was used (1.75 mg/kg), it was found that *d*-amphetamine substituted for the opioid cue. Thus, it may be that when animals are trained to discriminate higher doses of opioids from saline, activation of midbrain DA neurons by mu opioid receptor agonists is not the cue used by the animal to discriminate the presence of opioid drugs. In contrast, dopaminergic activation may serve as a cue when animals are trained to discriminate low doses of opioids from saline. It should be noted, however, that this explanation cannot account for the finding that activation of the DA system by *d*-amphetamine did not substitute for the fentanyl cue at a very low training dose (0.005 mg/kg) in Colpaert et al. (7) study; nor can it explain the finding that DA antagonists, at doses that did not reduce response rate, did not block the heroin cue in animals trained with a low dose of heroin (0.3 mg/kg) (8). Clearly, other factors must determine which actions of mu opioids are used by the animal to make the discrimination between the presence or absence of the drug.

In conclusion, the present study indicates that restraint stress or corticosterone administration given in close temporal contiguity to drug decreases the sensitivity to the opioid cue. In addition, under the conditions of the present experiment, activation of opioid receptors in the VTA did not mimic the discriminative stimulus effects of systemically administered heroin. These results, as well as the conflicting reports concerning the brain site(s) involved in opioid discrimination, support the view that multiple brain sites are involved in the discriminative stimulus effects of opioid agonists (see 4).

ACKNOWLEDGEMENTS

We thank Demetra Rodaros and Douglas Funk for their expert technical assistance. Corticosterone-21-hemisuccinate was generously given to us by P.-V. Piazza. Methyl naltrexone was generously supplied by Boehringer Ingelheim, Germany. This research was funded by a grant to J.S. from the Medical Research Council of Canada (MA6678). Y.S. holds a Post Doctoral Fellowship from the Medical Research Council of Canada.

REFERENCES

1. Bjorklund, A.; Lindvall, O. Dopamine-containing systems in the CNS. In: Bjorklund, A.; Hokfelt, T., eds. Handbook of chemical neuroanatomy (vol. 2). Amsterdam: Elsevier; 1984:55–122.
2. Broocks, A.; Schweiger, U.; Pirke, K. M. Hyperactivity aggravates semistravation-induced changes in corticosterone and triiodothyronine concentrations in plasma but not luteinizing hormone and testosterone levels. Physiol. Behav. 48:567–569; 1990.
3. Brown, D. R.; Goldberg, D. I. The use of quaternary narcotic antagonists in opiate research. Neuropharmacology 24:181–191; 1984.
4. Colpaert, F. C. Discriminative stimulus properties of narcotic analgesic drugs. Pharmacol. Biochem. Behav. 9:863–887; 1978.
5. Colpaert, F. C. Drug discrimination: Methods of manipulation, measurement, and analysis. In: Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer-Verlag; 1987:341–372.
6. Colpaert, F. C.; Niemegeers, C. J. E.; Jannsen, P. A. J. Fentanyl and apomorphine: Asymmetrical generalization of discriminative stimulus properties. Neuropharmacology 15:541–545: 1976.
7. Colpaert, F. C.; Niemegeers, C. J. E.; Jannsen, P. A. J. Factors regulating drug cue sensitivity: The effect of training dose in fentanyl-saline discrimination. Neuropharmacology 19:705–713; 1980.
8. Corrigal, W. A.; Coen, K. M. Selective D1 and D2 dopamine antagonists decrease response rates of food-maintained behavior and reduce the discriminative stimulus produced by heroin. Pharmacol. Biochem. Behav. 35:351–355; 1990.
9. De Boer, S. F.; Koopmans, S. J.; Slangen, J. L.; Gugten, J. Effects of fasting on plasma catecholamine, corticosterone and glucose concentrations under basal and stress conditions in individual rats. Physiol. Behav. 45:989–994; 1989.
10. Devine, D. P.; Wise, R. A. Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. J. Neurosci. 14:1978–1984; 1994.
11. Devine, D. P.; Leone, P.; Pocock, D.; Wise, R. A. Differential involvement of ventral tegmental mu, delta and kappa opioid receptors in modulation of basal mesolimbic dopamine release: In vivo microdialysis studies. J. Pharmacol. Exp. Ther. 266:1236–1246; 1993.
12. Fowler, S. C.; Johnson, J. S.; Kalman, M. J.; Liou, J. R.; Wilson, M. C.; Hikal, A. H. In a drug discrimination procedure isolation-reared rats generalize to lower doses of cocaine and amphetamine than rats reared in an enriched environment. Psychopharmacology (Berlin) 110:115–118; 1993.
13. Gaiardi, M.; Bartoletti, M.; Bacchi, A.; Gubellini, C.; Babbini, M. Increased sensitivity to the stimulus properties of morphine in food deprived rats. Pharmacol. Biochem. Behav. 26:719–723; 1987.
14. Giralt, M.; Armario, A. Individual housing does not influence the adaptation of the pituitary-adrenal axis and other physiological variables to chronic stress in adult male rats. Physiol. Behav. 45: 477–481; 1989.
15. Grilly, D. M.; Gowans, G. C. Comparison of the effects of morphine and immobilization stress on discrimination performance in rats. Behav. Neurosci. 100:512–524; 1986.
16. Harrap, S. B.; Louis, W. J.; Doyle, A. E. Failure of psychosocial stress to induce chronic hypertension in the rat. J. Hypertens. 2: 653–662; 1984.
17. Jaeger, T. V.; van der Kooy, D. Morphine fails to produce discriminative effects in the ventral tegmental area. Soc. Neurosci. Abstr. 19:1612; 1993.
18. Jaeger, T. V.; van der Kooy, D. Morphine acts in the parabrachial nucleus, a pontine viscerosensory relay, to produce discriminative stimulus effects. Psychopharmacology (Berlin) 110:76–84; 1993.
19. Jaffe, J. H.; Martin, W. R. Opioid analgesics and antagonists. In: Gilman, A. G.; Rall, T. E.; Nies, A. S.; Taylor, P., eds. Goodman & Gilman's the pharmacological basis of therapeutics (8th ed.). New York: Pergamon Press; 1990:485–521.
20. Kalivas, P. W.; Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain. Res. Rev. 16:223–244; 1991.
21. Koek, W.; Slangen, J. L. The role of fentanyl training dose and of the alternative stimulus condition in drug generalization. Psychopharmacology (Berlin) 76:149–156; 1982.
22. Krynock, G. M.; Rosecrans, J. A. Morphine as discriminative stimulus: Role of periaqueductal gray neurons. Res. Commun. Chem. Pathol. Pharmcol. 23:49–60; 1979.
23. Kvetnansky, R.; Mikulaj, L. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. Endocrinology 87:738–743; 1970.
24. Locke, K. W.; Holtzman, S. G. Characterization of the discriminative stimulus effects of centrally administered morphine in the rat. Psychopharmacology (Berlin) 87:1–6; 1985.
25. Meyerhoff, J. L.; Kant, G. J.; Bunnel, B. N.; Mougey, E. H. Regulation of pituitary cyclic AMP, plasma prolactin, and POMC-derived peptide responses to stressful conditions. In: Chrousos, G. P.; Loriaux, L. D.; Gold, P. W., eds. Mechanisms of physical and emotional stress. New York: Plenum Press; 1988: 107–122.
26. Miczek, K. A. Tolerance to the analgesic, but not the discriminative stimulus effects of morphine after brief social defeat in rats. Psychopharmacology (Berlin) 104:181–186; 1991.
27. Pallegrino, L. J.; Pallegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1986.
28. Piazza, P. V.; Deminiere, J. M.; Maccari, S.; Le Moal, M.; Mormede, P.; Simon, H. Individual vulnerability to drug-self-administration: action of corticosterone on dopaminergic systems as possible pathophysiological mechanism. In: Willner, P.; Scheel-Kruger, J., eds. The mesolimbic dopaminergic system: From motivation to action. New York: Wiley; 1991:473–495.
29. Rosecrans, J. A.; Glennon, R. A. Drug-induced cues in studying mechanisms of drug action. Neuropharmacology 18:981–989; 1979.
30. Shaham, Y. Immobilization stress-induced oral opioid self-administration and withdrawal in rats: Role of conditioning factors and the effect of stress on "relapse" to opioid drugs. Psychopharmacology (Berlin) 111:477–485; 1993.
31. Shaham, Y.; Alvares, K.; Nespor, S. M.; Grunberg, N. E. Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacol. Biochem. Behav. 41:615–619; 1992.
32. Shaham, Y.; Kelsey, J. E.; Stewart, J. Temporal factors in the effect of restraint stress on morphine-induced behavioral sensitization. Psychopharmacology (Berlin) 117:102–109; 1995.
33. Shaham, Y.; Stewart, J. Exposure to mild stress enhances the reinforcement efficacy of intravenous heroin self-administration in rats. Psychopharmacology (Berlin) 114:523–527
34. Shannon, H. E.; Holtzman, S. G. Evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 198: 54–65; 1976.
35. Shannon, H. E.; Holtzman, S. G. Discriminitive effects of morphine administered intracerebral in the rat. Life Sci. 21:585–594; 1977.
36. Shannon, H. E.; Holtzman, S. G. Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rats. Psychopharmacology (Berlin) 61:239–244; 1979.
37. Shippenberg, T.S.; Herz, A.; Spanagel, R.; Bals-Kubik, R.; Stein, C. Conditioning of opioid reinforcement: Neuroanatomical and neurochemical substrates. Ann. NY Acad. Sci. 654:347–356; 1992.
38. Shoaib, M.; Spanagel, R. Mesolimbic sites mediate the discriminative stimulus effects of morphine. Eur. J. Pharmacol. 252:69–75; 1994.
39. 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.
40. Ukai, M.; Holtzman, S. G. Restricted feeding does not modify

discriminative stimulus effects of morphine in the rat. Pharmacol. Biochem. Behav. 79:201–203; 1988.
41. Valentino, R. J.; Herling, S.; Woods, J. H.; Medzihradsky, F.; Merz, H. Quaternary naltrexone: Evidence for the central mediation of discriminative stimulus effects of narcotic agonists and antagonists. J. Pharmcol. Exp. Ther. 217:652–659; 1981.
42. van Ree, J. M.; Smyth, D. G.; Colpaert, F. C. Dependence creating properties of lipotropin c-fragment (β-endorphin): Evidence for its internal control of behavior. Life Sci. 24:495–502; 1979.
43. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.
44. Wise, R. A.; Hoffman, D. C. Localization of drug reward mechanisms by intracranial injections. Synapse 10:247–263; 1992.
45. Young, A. M.; Masaki, M. A.; Geula, C. Discriminative stimulus effects of morphine: Effects of training dose on agonist and antagonist effects of mu opioids. J. Pharmacol. Exp. Ther. 261: 246–257; 1992.