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Supplemental morphine infusion into the posterior ventral tegmentum extends the satiating effects of self-administered intravenous heroin

S. Steidl [1,*], S. Myal [2], R.A. Wise

Intramural Research Program National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA

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ABSTRACT

Rats learn to self-administer intravenous heroin; well-trained animals lever-press at a slow and regular pace over a wide range of intravenous doses. The pauses between successive earned infusions are proportional to the dose of the previous injection and are thought to reflect periods of drug satiety. Rats will also self-administer opiates by microinjection directly into sites in the posterior regions of the ventral tegmentum. To determine if the pauses between self-administered intravenous injections are due to opiate actions in posterior ventral tegmentum, we delivered supplemental morphine directly into this region during intravenous self-administration sessions in well-trained rats. Reverse dialysis of morphine into the posterior ventral tegmentum increased the intervals between earned injections. The inter-response intervals were greatest for infusion into the most posterior ventral tegmental sites, sites in a region variously known as the tail of the ventral tegmental area or as the rostromedial tegmental nucleus. These sites at which morphine prolongs inter-response intervals, correspond to the sites at which opiates have been found most effective in reinforcing instrumental behavior.

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1. Introduction

The reinforcing effects of opiates are thought to rely primarily on their ability to activate opioid receptors in the ventral tegmentum. Rats quickly learn to self-administer morphine (Bozarth and Wise, 1981a; Welzl et al., 1989; Devine and Wise, 1994) and mu (Devine and Wise, 1994; Zangen et al., 2002) and delta (Devine and Wise, 1994) opioids into the ventral tegmental area (VTA), and into a region just caudal to it identified by some as the "tail" of the VTA (tVTA: (Perrotti et al., 2005)) and by others as the rostromedial tegmental nucleus (RMTg: (Jhou et al., 2009)). Rats learn to self-administer intravenous heroin (di-acetyl morphine) which is metabolized to morphine as it crosses the blood–brain barrier. Morphine then acts, presumably in this region to inhibit GABAergic neurons that normally hold VTA dopamine neurons under inhibitory control (Jhou et al., 2009; Johnson and North, 1992; Margolis et al., 2014). Heroin is thought to be more addictive than its metabolite morphine because it crosses the blood–brain barrier more readily than morphine (Oldendorf et al., 1972); when heroin is injected, however, it is the metabolite, morphine, that binds to opioid receptors, disinhibits the dopamine system, and activates the reward system (Bozarth and Wise, 1981a; Phillips and LePiane, 1980).

Rats self-administer heroin and psychomotor stimulants intravenously, and this behavior is characterized (over the working range of doses per injection) by regular inter-response intervals that reflect the time to metabolize what has already been taken (Dougherty and Pickens, 1974; Gerber and Wise, 1989; Yokel and Pickens, 1974). The spacing of the injections appears to reflect periods of drug satiety (Wise, 1987). In the present study we sought to determine if the periods of apparent satiety could be increased by infusions of morphine directly into sites where the drug is thought to have its primary rewarding effects. Thus we assessed the temporal pattern of responding for intravenous heroin in well-trained rats following reverse dialysis of morphine or artificial cerebrospinal fluid (aCSF) into a range of ventral tegmental sites.

2. Materials and methods

2.1. Animals and surgery

Thirteen male Long–Evans rats (Charles River, Raleigh, NC) weighing 275–325 g at the time of surgery were used. Each rat was individually housed under a reverse light-dark cycle (12/12, lights off at 8 am) with free access to food and water. All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the NIDA Intramural Research Program. Each rat was anesthetized first with a combination of ketamine and xylazine (57 mg/kg and 9 mg/kg i.p., respectively). Anesthesia was then maintained during the surgery with isoflurane

* Corresponding author at: Department of Psychology, Loyola University Chicago, 1032 West Sheridan Road, Chicago, IL 60626, USA. Tel.: +1 773 508 2971; fax: +1 773 508 8713.
E-mail address: ssteidl@luc.edu (S. Steidl).
[1] Current Address: Department of Psychology, Loyola University Chicago, IL 60626, USA.
[2] Current Address: Medical Scientist Training Program, University of Pittsburgh, PA 15261, USA.

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(2–3% in 1 L/min oxygen). An intravenous microrenathane catheter (Braintree Scientific; Braintree, MA) was first inserted into the right external jugular vein. Catheter tubing was attached to a cannula adaptor fixed to the rat's skull. Catheters were flushed daily with heparin (10 USP/ml in sterile saline), containing gentamicin (0.08 mg/ml).

Each rat was also implanted, during the same surgery, with bilateral guide cannulae (CMA-11) for microdialysis. To avoid puncture of the midsagittal sinus, guide cannulae were angled at 12° the midline. Guide cannulae were aimed at each of three levels of the ventral tegmentum: anterior VTA (AP −5.0, ML ±2.4, DV −8.0), the posterior VTA (AP −6.00, ML ±2.1, DV −7.6) or the tVTA/RMTg (AP −6.8, ML ±2.3, DV −7.4; Paxinos and Watson, 2007).

2.2. Drugs

Ketamine, xylazine, and 3,6-diacetylmorphine hydrochloride (heroin), and morphine sulfate were obtained from the NIH. Heroin was dissolved in 0.9% sterile saline. Morphine sulfate was dissolved in artificial cerebrospinal fluid (aCSF).

2.3. Heroin intravenous self-administration

2.3.1. Apparatus

Operant conditioning chambers, measuring 25 × 27 × 30 cm were used. Each chamber was equipped with two response levers (one retractable lever and a stationary), a red house light, and a white cue light located above the retractable lever. Chambers were housed within sound-attenuating enclosures equipped with fans that provided both ventilation and a constant source of masking noise.

2.3.2. Drug self-administration procedure

Each rat was housed and trained in a dedicated operant chamber throughout the experiment with food and water available ad libitum. Daily 4-hour intravenous heroin self-administration training sessions began 7–10 days after i.v. catheter and guide cannulae implantation and lasted for 13–15 days. For each rat the head-mount catheter was connected to an infusion line attached to a liquid swivel that allowed for free movement of the rat within the operant chamber. The beginning of each daily training session was signaled by insertion of the retractable response lever into the operant chamber. For the first 7 training sessions each rat was allowed to self-administer intravenous heroin at 0.1 mg/kg/infusion under a fixed ratio 1 schedule of reinforcement with a 20-s time-out period accompanied by illumination of a white cue light located above the retractable lever. Lever presses during the time-out period were recorded but were not reinforced. Presses on the stationary lever were recorded but had no programmed consequence. At the end of the training session the retractable lever was withdrawn from the chamber and the house light was turned off. For training sessions 8–12 and for training sessions 13–15 each rat was allowed to self-administer 0.05 and 0.025 mg/kg/infusions, respectively.

2.4. Morphine reverse dialysis

Immediately following the last training session rats were lightly anesthetized with isoflurane and microdialysis probes with 1 mm active membrane (CMA 11) were bilaterally inserted into the respective brain sites. The probes were continuously perfused with artificial cerebrospinal fluid (aCSF: composition in mM: NaCl, 148; KCl, 2.7; $CaCl_2$, 1.2; $MgCl_2$, 0.8, pH 7.4) at a rate of 0.5 μl/min overnight. The flow rate was increased to 2 μl/min 1 h prior to the beginning of the testing session on the next day. On each of the following two days, the rats were allowed to self-administer heroin (0.025 mg/kg/infusion) in 6-h sessions. During the first 2 h of each session the rats received aCSF by reverse dialysis. During the next 4 h of each session, the rats either continued to receive aCSF or received 10 mM morphine by reverse dialysis; the opposite treatment was given on the second day with sequence counterbalanced across animals. Flow rate was decreased to 0.5 μl/min between sessions and was restored to 2 μl/min 1 hour prior to the second session. The 10 mM dose of morphine was chosen as we found little effect of a 1 mM morphine dose on intravenous heroin self-administration in pilot studies.

2.5. Histology

Following completion of behavioral testing, each rat was deeply anesthetized with a mixture of pentobarbital (30 mg/kg, i.p.) and chloral hydrate (140 mg/kg, i.p.) and transcardially perfused with 0.1 M phosphate buffer (PB) followed by 4% (*W*/*V*) paraformaldehyde in 0.1 M PB, pH 7.3. The brains were removed and left in 4% paraformaldehyde for 2 h at 4 °C and then transferred to an 18% sucrose solution in PB. Coronal sections (40 μm) were prepared for each rat and mounted on to gelatin-coated slides. Sections were stained with cresyl violet and microdialysis probe locations were examined using a light microscope.

2.6. Data analysis

Intravenous heroin self-administration data were analyzed for the self-administration training period using repeated-measures analysis of variance (ANOVA) with the i.v. heroin DOSE (0.1, 0.05, 0.025 mg/kg/inf) as the only factor. Significant main effects were further analyzed using Fisher's LSD post-hoc test. The relation between probe distance (mm caudal from bregma) and i.v. heroin intake for each time period following reverse dialysis of morphine was analyzed by ANOVA and by correlational analysis. In the ANOVA, intended stereotaxic PLACEMENT (aVTA, pVTA, RMTg) and TIME (the first 2 hour [0–120 min] baseline period, and the last 3 hour [180–360 min] period of stable responding) were included as between-subjects and within-subjects factors, respectively. The 1-hour transition period (120–180 min) was not included in the analysis on the basis of our previous findings (Suto and Wise, 2011) that it takes a similar period for the effects on intravenous cocaine self-administration to stabilize when dopamine agonists are infused into nucleus accumbens by reverse dialysis.

3. Results

3.1. Histology

The dialysis probes were distributed throughout the rostro-caudal extent of the ventral tegmentum, ranging from AP −4.80 to AP −7.08 (Fig. 1A). Placements are reconstructed in sagittal section in Fig. 1B.

3.2. Heroin self-administration training

Rate of responding became reliable within a few days of training and was inversely related to dose per injection (Fig. 2A; main effect of DOSE [$F_{2, 24} = 35.57$, $p < 0.00001$]; Fisher's LSD post-hoc test showed greater numbers of infusions at the 0.05 mg/kg/inf dose relative to the 0.1 mg/kg/inf dose [$p < 0.0001$] and at the 0.025 mg/kg/inf dose relative to the 0.1 mg/kg/inf dose [$p < 0.000001$] and the 0.05 mg/kg/inf dose [$p < 0.05$]). All rats showed stable levels of heroin self-administration (<10% variation in daily intake) by the end of the training period, making approximately 6 responses per hour for the dose of 0.025 mg/kg/injection.

3.3. Intracranial reverse dialysis of morphine

Response rates became unstable over the first hour of continuous morphine, but not aCSF, infusion (Fig. 2B and D) and restabilized, again becoming linear and settling at mean rates of 2.5 or less in the last 3 h of testing (Fig. 2B). This was reflected in a main effect of TIME ($F_{59, 590} = 58.08$, $p < 0.000001$) in an analysis of variance comparing