Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat

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Abstract. A quaternary derivative of naloxone, methyl naloxonium chloride (MN), was administered intracerebrally to rats trained to self-administer heroin intravenously. Increases in intravenous (IV) heroin self-administration rates were found following injections of low doses of MN into the nucleus accumbens (N.Acc), but not following injections of low doses of MN into the ventral tegmental area (VTA). These results were interpreted to suggest that the rewarding properties of IV heroin were decreased following N.Acc opiate receptor blockade. The relative insensitivity of the VTA to MN treatment was taken to suggest that VTA opiate receptors are either not essential or play a secondary role in mediating IV heroin self-administration. The present data support the notion that post-synaptic N.Acc opiate receptors play a crucial role in maintaining IV heroin self-administration.

Key words: Heroin – Intravenous self-administration – Methyl naloxonium chloride – Opiates – Reward – Nucleus accumbens – Ventral tegmental area – Rat

Studies employing intravenous (IV) self-administration techniques have shown that opiate antagonists block the reinforcing properties of opiate drugs (Goldberg et al. 1971; Weeks and Collins 1976; Ettenberg et al. 1982; Koob et al. 1984). Further, recent evidence has shown that the attenuation of heroin reward depends solely on the central opiate receptor blocking properties of the antagonist (Koob et al. 1984; Vaccarino et al. 1985). Vaccarino et al. (1985) found that intracerebroventricular administration of methyl naloxonium chloride (MN) (quaternary naloxone analog), an opiate antagonist which does not readily cross the blood-brain barrier (Bianchi et al. 1982; Russell et al. 1982; Koob et al. 1984), attenuates heroin reward during IV heroin self-administration in doses which are ineffective when injected systemically (Koob et al. 1984). These findings raise the possibility that localized MN injections into discrete brain regions can be used as a technique to map out central opiate receptor sites critical for IV opiate self-administration.

While the opiate receptor sites underlying IV opiate self-administration are unknown, recent studies have shed light on possible sites mediating the locomotor-activating properties of the opiates. It has been found that microinjections of morphine or D-Ala-methionine enkephalinamide (DALA) (a peptidase-resistant synthetic enkephalin analogue) into either the ventral tegmental area (VTA) or nucleus accumbens can produce an increase in locomotor activity (Pert et al. 1976; Pert and Sivit 1977). More recently, Kalivas et al. (1983) have provided evidence suggesting that while the locomotor activating properties of intra-VTA DALA injections are dependent on mesolimbic dopamine (DA) function, the locomotor activating effects of intra-N.Acc DALA injections are independent of mesolimbic DA. It was reported that intra-N.Acc injections of fluphenazine (a DA antagonist) or mesolimbic 6-OHDA lesions do not block the locomotor activating properties of intra-N.Acc DALA injections (Kalivas et al. 1983). This is in contrast to the finding that intra-N.Acc injections of neuroleptics or mesolimbic 6-OHDA lesions both antagonize the locomotor activation produced by intra-VTA injections of DALA (Kelley et al. 1980). Together, these findings suggest that the behavioral activating properties of opiates can be mediated by opiate receptors in either the VTA (via the mesolimbic DA system) or in the N.Acc (independent of the mesolimbic DA system).

Based on the suspected overlap in the neural substrates mediating drug-induced locomotor activation and reward (Pert et al. 1976; Kelley et al. 1980; Ettenberg et al. 1982; Fink and Smith 1980; Vaccarino and Franklin 1982; Koob and Bloom 1983; Kalivas et al. 1983), these findings raise the possibility that VTA and/or N.Acc opiate receptors are involved in the mediation of IV heroin reward. Therefore, the present study investigated the role of VTA and N.Acc opiate receptors in IV heroin self-administration. To this end, the effects of intra-VTA and intra-N.Acc injections of the opiate antagonist MN on IV heroin self-administration were tested. Direct involvement of either of these two sites in IV heroin reward would thus be expected to result in blockade of the reinforcing properties of IV heroin following MN injections as reflected in an increase in heroin self-administration (Ettenberg et al. 1982; Koob et al. 1984). This change in response rate is considered to reflect a compensatory increase in responding due to the decreased effectiveness of the reinforcer (e.g., Glick et al. 1975).

Materials and methods
Heroin self-administration

The subjects were 15 male, albino, Wistar rats (Charles River Laboratories) weighing 250–300 g at the start of the
experiment. Each rat was first trained to lever press for food. Rats were food deprived for 24 h and then exposed to an operant chamber where a perforated lever filled with food pellets was available. When depressed, this lever delivered a 45-mg Noyes pellet on a continuous reinforcement schedule. Each rat was allowed to self-train and press for 100 pellets before being returned to free feeding.

Following acquisition of lever pressing for food, rats were surgically implanted with bilateral 23 gauge stainless steel cannulae aimed 3 mm dorsal to the VTA (n = 7) or N.Acc (n = 8). Coordinates (Pellegrino et al. 1979) were: VTA, 3.0 mm posterior to bregma, ±1.1 mm lateral to the midline; 5.7 mm ventral to the skull surface; N.Acc, 3.2 mm anterior to bregma, ±1.7 mm lateral to the midline, 5.8 mm ventral to the skull surface. Following surgery a 30 gauge dummy cannula was inserted into the guide cannula so that the base was flush with the base of the guide cannula. During injections 30 gauge needles protruded 3 mm from the guide cannula base.

In addition to being implanted with bilateral cannulae, rats received chronic silastic jugular cannula implants (Roberts et al. 1977, 1980). The jugular cannula was passed subcutaneously to a poly-ethylene assembly mounted on the animal’s back. This assembly consisted of a Plastic Products guide cannula (C313G) which was bent at a right angle half-way down the cannula. The junction was then glued (Super Glue) and the guide cannula embedded into a 1 in square piece of marlex mesh with epoxy. The dorsal and ventral junctions of the marlex mesh/guide cannula were covered with silicon rubber adhesive sealant (General Electric). The marlex mesh was then sutured into the rat’s back. A stylet was inserted into the guide cannula which protruded from the rat’s back. All surgery was carried out under 50 mg/kg sodium pentobarbital anesthesia.

For self-administration testing, a cannula connector with a spring cover (c313c, Plastic Products), which was connected to a swivel and syringe pump as described by Roberts et al. (1977, 1980), was screwed into the guide cannula mounted on the animal’s back immediately prior to the beginning of each session. The cannula connector was removed following self-administration sessions.

The animals lived for the duration of the experiment inside individual standard operant-conditioning cages where they had free access to food and water. The cages themselves were housed inside sound attenuated chambers and maintained on a 12-h reversed light-dark cycle (lights off from 9:00 a.m. to 9:00 p.m.).

Heroin self-administration testing began a minimum of 2 days post-surgery. Each rat was allowed 3 h access every day (commencing in the 1st h after lights out) to a metal lever mounted on the front wall of its cage. A lever-press resulted in an IV injection of 0.1 ml heroin (0.75 mg/kg/injection) dissolved in 0.9% physiological saline and administered over a period of 4 s. A signal light mounted above the lever indicated the onset of an injection and remained lit for 20 s, during which time the lever was inactive. Lever-presses during the period when the signal light was not lit were reinforced under a schedule of continuous reinforcement.

Three to 5 days following acquisition of lever pressing for IV heroin (minimum of ten lever presses for the total 3-h session) rats were treated intraperitoneally with naloxone (0.6 mg/kg) 15 min prior to the self-administration test. MN testing began following 3 consecutive days of stable responding (±10% of average) after the systemic naloxone tests. VTA rats were pre-treated with intra-VTA injections of 0.0, 0.5 and 1.0 µg MN. N.Acc rats were pre-treated with intra-N.Acc injections of 0.0, 0.125, 0.25 and 0.5 µg MN. A minimum of 3 no pre-treatment days separated drug test days during which time rats continued to have daily 3-h access to IV heroin. In order to avoid possible carryover effects of high doses of MN, MN doses were administered in ascending order. The dose ranges were chosen based on pilot studies showing that 0.25 and 0.5 µg MN produced equipotent increases in responding for IV heroin when injected into the N.Acc, but had no effect when injected into the VTA.

Following MN testing, heroin self-administration sessions were terminated. Three days after the last heroin self-administration session, five N.Acc rats (all of which had shown increases in responding following following intra-N.Acc MN treatment) began training for IV cocaine self-administration. A lever press resulted in an IV injection of 0.1 ml cocaine hydrochloride (0.75 mg/kg/injection). All other aspects of the self-administration method were the same as those described for heroin. Following 3 consecutive days of stable responding for cocaine each of the five rats was pre-treated with an intra-N.Acc injection of 0.5 µg MN. Intra-N.Acc injection methods were the same as those already described.

Following testing, all rats were sacrificed with an overdose of pento-barbital and perfused transcardially first with saline and then with 10% formalin. Injection cannulae tip placements were verified from 40 µm thionin-stained sections.

**Drugs**

The drugs used in this experiment were heroin and cocaine hydrochloride (generously provided by the National Institute of Drug Abuse), naloxone hydrochloride (generously provided by Endo Laboratories, Inc.). MN (ORG 10908) was generously provided by Dr. Joop de Graaf of Organon, Inc., OSS Holland. All drugs were dissolved in 0.9% sterile saline for injection. Naloxone was administered in a volume of 1 ml/kg and MN was administered in a 2 µl volume (1 µl per side) over a period of 2 min using a Harvard Apparatus Compact Infusion Pump. MN was administered 10 min prior to the start of the self-administration session.

**Results**

**Histological results**

Histological analyses revealed that of the eight rats with injection cannulae aimed at the N.Acc, seven had tips located bilaterally within the N.Acc. The remaining rat had the injection tips located ventral to the N.Acc and was therefore not included in the analyses. Six of the seven VTA rats had injector tips located bilaterally in the VTA. The remaining rat had the injector tips located dorsal to the VTA, and was therefore not included in the analyses (See Fig. 1).

As in previous studies (Koob et al. 1982; Vaccarino et al. 1985), rats generally required 5–7 days to acquire the lever pressing response for IV heroin (defined as a minimum of ten responses per 3-h session). Following acquisition rats typically required an addition 8–12 days to reach stable