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BRIEF COMMUNICATION

Effects of Intracerebroventricular Administration of Methyl Naloxonium Chloride on Heroin Self-Administration in the Rat

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VACCARINO, F. J., H. O. PETTIT, F. E. BLOOM AND G. F. KOOB. *Effects of intracerebroventricular administration of methyl naloxonium chloride on heroin self-administration in the rat.* PHARMACOL BIOCHEM BEHAV **23**(3) 495–498, 1985.—A quaternary derivative of naloxone, methyl naloxonium chloride (MN), was administered intracerebroventricularly (ICV) to rats trained to self-administer heroin intravenously. MN produced a dose-dependent (0.5–4.0 μg) increase in responding for heroin. Since MN is unlikely to reach the peripheral circulation in these doses, these results were viewed as strongly supporting the hypothesis that central opiate receptors are solely responsible for mediating the reinforcing properties of heroin during self-administration. In addition, the present study suggests that intracerebral MN injections may prove to be a useful technique in the search for central substrates of heroin reward.

Methyl naloxonium chloride Heroin Intravenous Self-administration

STUDIES employing intravenous (IV) self-administration techniques have shown that opiate antagonists block the reinforcing properties of opiate drugs [3, 5, 8, 12]. Recent evidence has further suggested that this attenuation of heroin reward may depend solely on the central opiate receptor blocking properties of the antagonist. Koob *et al.* [6] found that while peripherally administered naloxone blocked the reinforcing properties of IV heroin, peripherally administered methyl naloxonium chloride (MN), a quaternary derivative of naloxone, had little effect. Both drugs injected peripherally have peripheral opiate antagonist properties [1,11]. However, only naloxone appears to act centrally [1, 2, 11]. Therefore, these results suggested that blockade of central opiate receptors is necessary to block the reinforcing properties of IV heroin self-administration.

While the latter results indicate that central opiate receptors are important in the mediation of heroin reward, they do not exclude the possibility that both central and peripheral opiate receptors must be blocked in order to attenuate heroin reward during self-administration. A more direct way of assessing the role of central opiate receptors would be to investigate the effects of direct central injections of an opiate antagonist, such as MN, on IV heroin self-administration. Since MN does not appear to readily cross the blood brain barrier [1, 6, 11], any antagonistic effects observed following central administration of MN could be attributed to its central opiate receptor blocking properties. To date no studies have investigated the effects of direct central injections of MN. Thus, the present study examined the effects of direct intracerebroventricular (ICV) injections of MN on rats trained to respond to IV heroin reinforcement. In this way, the feasibility of using central MN injections as a technique to block the reinforcing properties of heroin could be tested.

Blockade of the reinforcing properties of heroin in our self-administration model is reflected in an increase in responding, particularly during the first part of the daily session [3,6]. This change in responding closely resembles extinction with higher doses of the antagonist and is characterized by an increase in the rate of self-administration similar to that observed following a decrease in the injection dose of heroin [4].

METHOD

Heroin Self-Administration

The subjects were six male, albino, Wistar rats weighing 300–350 g at the start of the experiment. Each rat was first trained to lever press for food. Rats were food deprived for

24 hours and then exposed to an operant box 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 ad lib food.

Following acquisition of lever pressing, rats were surgically implanted with a 23 gauge stainless steel ICV cannula aimed 1 mm dorsal to the right or left lateral ventricle. Coordinates [7] were: 0.6 mm posterior to bregma; ±2.0 mm lateral to the midline; 3.2 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. Injection needles (30 gauge) protruded 1 mm out of the guide cannula base.

In addition to being implanted with an ICV cannula, rats received chronic silastic jugular cannula implants [9,10]. The jugular cannula was passed subcutaneously to a polyethylene assembly mounted on the animal's back. This assembly consisted of a Plastic Products guide cannula (C313G) which was bent at a right angle halfway down the cannula. The junction was then glued (Super Glue) and the guide cannula embedded into a one square inch 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 dummy cannula 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.* [9,10] 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 were provided with ad lib access to food and water. The cages themselves were housed inside sound attenuated chambers and maintained on a 12-hour reversed light-dark cycle (lights off from 9:00 a.m. to 9:00 p.m.).

Heroin self-administration testing began a minimum of two days post-surgery. Each rat was allowed 3-hour access every day (commencing in the first hour after lights out) to a metal lever mounted on the front wall of its cage. The lever was mounted in the cage wall at the beginning of each session and removed at the end. A lever-press resulted in a IV injection of 0.1 ml of heroin (0.06 mg/kg injection) dissolved in 0.9% physiological saline and administered over a period of 4 seconds. A signal light mounted above the lever indicated the onset of an injection and remain lit for 20 seconds, during which time the lever was inactive. Lever-presses during the period when the signal light was not lit were reinforced on a schedule of continuous reinforcement. At the beginning of each session rats were given two consecutive IV heroin injections delivered by the experimenter.

Three to five days following acquisition of lever pressing (i.e., minimum of 10 lever presses for the total 3-hour session), all rats were treated intraperitoneally with naloxone (1 mg/kg) 15 minutes prior to the self-administration test. MN testing began following three consecutive days of stable responding (±10% of average) after naloxone tests. Each rat (N=6) was pre-treated with ICV injections of 0, 0.5, 1.0, 2.0 and 4.0 μg of MN 10 minutes prior to the start of the self-administration session. Each dose level of MN was tested once. A minimum of three no pre-treatment days separated drug test days, during which time rats continued to have daily 3-hour access to IV heroin. In order to avoid possible carryover effects of high doses of heroin and MN, doses were administered in ascending order. Injections were made using gravitational force. The dose range was chosen on the basis of pilot studies showing that 4, 8, and 16 μg MN administered ICV produced equipotent increases in responding.

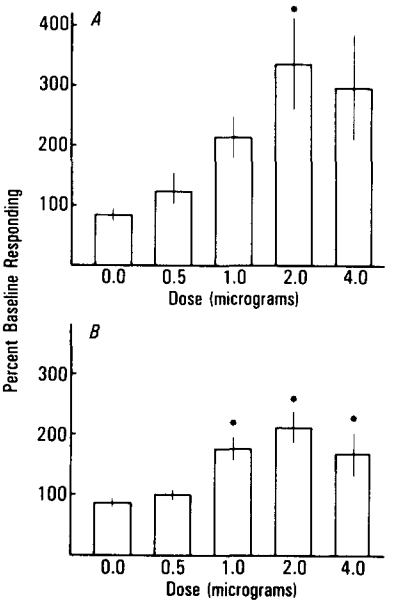

FIG. 1. The effects of ICV MN treatment on responding for heroin over the first hour (A) and over the total three-hour self-administration session (B). Response rates are expressed as the percentage of the individual subjects' baseline responding. Asterisks indicate that the treatment dose was significantly different from the saline treatment, $p<0.05$, Newman-Keuls test. Six rats were tested across all drug treatments. The day prior to ICV injections was used as the baseline day. The mean±S.E.M. baseline responding for the three-hour session was 19.2±2.4. There was no significant difference in baseline responding across days, $F(4,24)<1.0$, N.S.

Drugs

The drugs used in this experiment were heroin hydrochloride (generously provided by the National Institute of Drug Abuse) and naloxone hydrochloride (generously provided by Endo Laboratories, Inc.). MN (ORG 10908) was generously provided by Dr. Joop de Graaf of Organon. 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 1 μl volume.

RESULTS

All six rats achieved criteria. In the present paradigm, rats generally required 5 to 7 days to acquire the lever pressing response for IV heroin (minimum 10 responses per 3-hour session). Following acquisition, rats typically required an additional 8 to 12 days to reach stable responding (defined as ±10% of the average over three consecutive days of responding).

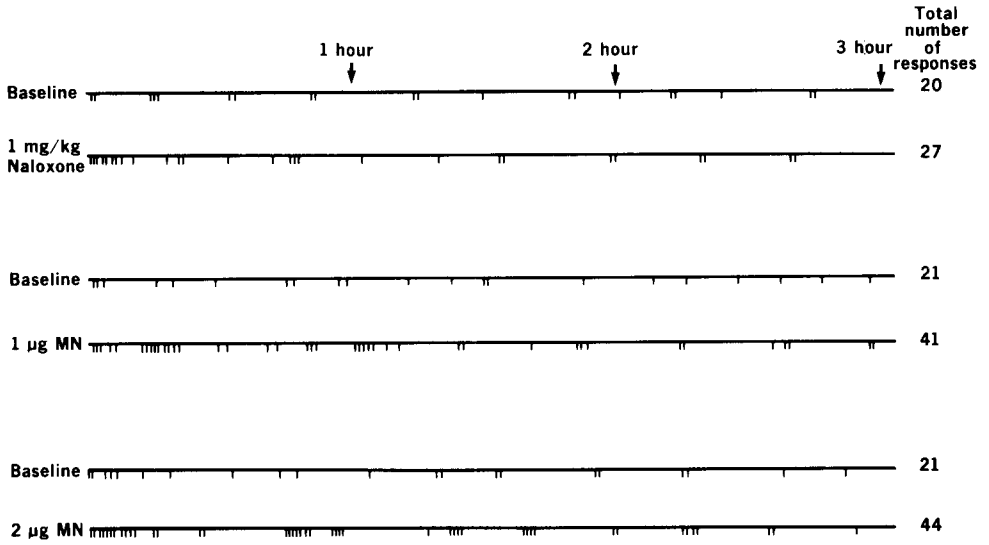

FIG. 2. IV heroin self-administration records for a representative rat following treatment with: naloxone (1 mg/kg IP), MN (1 μg ICV) and MN (2 μg ICV). Baseline titration records represent response patterns on the day prior to treatment with the antagonist. Note the similarity in the extinction-like pattern of responding following treatment with naloxone and MN. Total response rates are noted in the right-hand column.

During the first hour of each baseline heroin self-administration session, rats respond at relatively high rates, then tend to maintain a stable drug intake for the duration of the session. Consistent with the effects of peripherally administered naloxone [6], MN produced a dose-dependent increase in the loading dose during the first hour of responding, $F(4,24)=4.280$, $p=0.012$ (Fig. 1A). This increase in responding peaked at the 2 μg dose. Individual means comparisons with saline-treated rats revealed significant increases at the 2.0 μg dose, $p<0.05$, Newman-Keuls test.

The results for the total session were similar to those described for the first hour (Fig. 1B). At low doses, MN produced a dose-dependent increase in responding which peaked at the 2 μg dose, $F(4,24)=5.811$, $p=0.003$. Individual means comparisons with saline treatment revealed significant increases at 1.0, 2.0 and 4 μg doses, $p<0.05$, Newman-Keuls test. Figure 2 shows the response pattern of a representative rat following treatment with naloxone (1 mg/kg), MN (1 μg) and MN (2 μg).

DISCUSSION

The present study is consistent with previous reports showing that naloxone dose-dependently decreases the reinforcing value of heroin [3, 5, 6, 12]. Moreover, since peripherally administered MN in the present dose range has no effect on IV heroin self-administration [6], the present findings suggest that MN exerts its effects centrally. These data are consistent with previous findings indicating that blocking central opiate receptors is sufficient to block the reinforcing value of IV heroin administration.

It is important to note that blocking the reinforcing properties of opiate drugs in the present operant paradigm is reflected behaviorally as an increase in the rate of self-administration following treatment with opiate receptor antagonists [3, 5, 6, 12]. This is consistent with other studies and has been viewed as a compensatory change in operant behavior in response to the decreased effectiveness of the opiate as a reinforcer (due to receptor occupancy by the antagonist). This behavioral change is analogous to the increase in the rates of IV opiate administration observed following a decrease in the injection dose [4].

While our results suggest that MN acts centrally to block heroin reinforcement as reflected by an increase in responding, the neurochemical and anatomical substrates underlying the present effects remain subjects for future research. The present study, however, suggests that intracerebral MN injections may prove to be a useful technique in the search for central substrates of heroin reward.

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