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Low Doses of Methylnaloxonium in the Nucleus Accumbens Antagonize Hyperactivity Induced by Heroin in the Rat

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AMALRIC, M. AND G. F. KOOB. *Low doses of methylnaloxonium in the nucleus accumbens antagonize hyperactivity induced by heroin in the rat.* PHARMACOL BIOCHEM BEHAV **23**(3)411–415, 1985.—The effect of microinjection of a quaternary opiate antagonist methylnaloxonium (MN) in the lateral ventricle, ventral tegmental area (VTA) and nucleus accumbens (N.Acc.) was examined on the locomotor activation produced by a subcutaneous heroin injection (0.5 mg/kg) in the rat. At this dose heroin typically produced an initial depressant phase (0–30 min) followed by a prolonged hyperactivity (40–120 min). Lateral ventricular injections did not significantly reverse the initial depressant effects of heroin (0–30 min), and a dose of 4 μg was needed to reverse the subsequent hyperactivity (40–120 min). The most potent blockade was observed following injections into the N.Acc. where 0.1 μg significantly reversed the initial depressant effects of heroin (0–30 min), and 0.25 μg significantly reversed the subsequent hyperactivity (40–120 min). In the VTA, MN had the weakest effects, failing to reverse significantly the initial depressant effects of heroin (0–30 min), and only attenuating the subsequent hyperactivity at the highest doses. It is suggested that certain opiates act on the mesolimbic dopaminergic pathway at the level of the cell bodies in the VTA, but more critically in the N.Acc., possibly on opiate receptors postsynaptic to the dopamine neurons.

Methylnaloxonium Ventral tegmental area Nucleus accumbens Locomotor activity Rat Heroin Opiates

THE concentration of opiate receptors through which opiates and endogenous morphine-like compounds (opioid peptides) produce their pharmacological effects has been shown to vary in different brain regions [1, 2, 16]. Dopaminergic areas in brain like the striatum and the mesolimbic DA system appear to be characterized by a high density of such receptors [1, 2, 18]. The mesolimbic dopaminergic system whose cell bodies are localized in the ventral tegmental area (VTA) and project to the nucleus accumbens (N.Acc.) is known to have important functions in exploratory and locomotor behaviors and the behavioral activation associated with psychomotor stimulants such as amphetamine [7,13].

Several studies have investigated the role of the mesolimbic DA system in the behavioral activation induced by opiates [9] and opioid peptides [4, 8, 10, 11, 12, 15, 21]. Opiates, systemically injected, are known to produce at low doses analgesia and hyperactivity, and at higher doses, a state of immobility followed by a gradual increase in locomotor activity [3]. This opiate-like behavioral activation can be reproduced by intraventricular injection of opioid peptides [19]. A variety of behavioral studies have provided evidence that this locomotor activation is due to a stimulation of the mesolimbic dopamine system. Microinjections of morphine, D-ala-enkephalin analogs (DALA) and β-endorphin directly into the ventral tegmental area of the rat brain produce an increase in spontaneous motor activity resembling that observed with systemic injections of opiates [4, 9, 10, 11, 12, 21]. Furthermore, neurochemical studies have reported that some opiate receptors, identified by [^{3}H]naloxone, are located on dopaminergic terminals in the nucleus accumbens [2,18]. Other authors have focused their studies on the role of the nucleus accumbens in the locomotor activation induced by opiates and opioid peptides [5, 11, 15]. They have shown that direct administration of morphine or DALA into the nucleus accumbens produced an increase in locomotor activity. The excitatory effects of morphine were antagonized by systemic injections of naloxone (an opiate receptor antagonist) but not by haloperidol (a dopaminergic antagonist) [15]. In addition, 6-hydroxydopamine destruction of the DA terminals in the N.Acc. failed to block the locomotor activating effects of opioid peptides; in fact there appears to be a "cross-supersensitivity" [11].

The present study was designed to examine the specific interactions of opiates with mesolimbic DA system, either at the level of the cell bodies (VTA) or postsynaptically in the nucleus accumbens. The effects of subcutaneously injected heroin on locomotor activity were measured in photocell

cages. The respective influence of the VTA or the nucleus accumbens on this activation was examined by injecting intracerebrally in rats a quaternary opiate antagonist, methylnaloxonium (MN), which does not cross the blood brain barrier. Effects of MN into the lateral ventricle was first studied (Experiment 1). MN was also injected into either the ventral tegmental area (Experiment 2) or nucleus accumbens (Experiment 3) and the resulting blockade of locomotor activation induced by heroin injection was then measured.

METHOD

Subjects

The subjects were 125 male albino Wistar rats, weighing 250–270 g at the start of the experiment. They were housed in cages of 3 and maintained in a temperature and light controlled environment (7 a.m.–7 p.m. hours light). Food and water were available ad lib. Behavioral testing was always done on mornings 9 a.m. to 2 p.m., during the light period.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg IP). The study was divided into 3 experiments. In Experiment 1, rats were stereotaxically implanted with stainless steel cannulae 7 mm long (23 gauge) unilaterally 1 mm above the lateral ventricle (n=41). Coordinates: −0.6 mm posterior to the bregma, 2 mm lateral and 3.2 mm below skull surface at the point of entry. In Experiment 2, cannulae (10 mm) were bilaterally placed 3 mm above the VTA (n=56): +3.0 mm anterior to ear bar zero, 1.1 mm lateral and −5.7 mm from the skull surface. In Experiment 3, cannulae (10 mm) were bilaterally aimed at 2 mm above the nucleus accumbens (n=28): 3.2 mm anterior, 1.7 mm lateral and −5.8 mm ventral from the bregma. Stereotaxic coordinates were taken with the incisor bar 5.0 mm above the interaural line. All animals were allowed one week recovery before the start of behavioral testing.

Injection Procedure

Different doses of methylnaloxonium (MN): 0.1, 0.25, 1.0, 2.0, 4.0 μg/2 μl were prepared by addition of 0.9% saline for the injections. For the intracerebroventricular injections a 30 gauge stainless steel injection needle with 30 cm of tubing attached was inserted through the guide to 1 mm beyond its tip. Two μl of peptide was injected by gravity over a 30 sec period by raising the tubing above the head of the rat until flow began. The doses tested were 0.25, 1.0, 2.0, 4.0 μg in 2 μl of solution. For the intracerebral infusion procedure, injection needles (13 mm) were lowered into the VTA and bilateral infusion of 1 μl of solution was made over 2 minutes, using a microdrive pump. The injectors were left in place for 1 min to allow for diffusion. Before testing, all rats were given a preliminary saline infusion into the VTA in order to habituate them to the procedure and also to prevent for possible mechanical effects during experimental infusions. The same procedure was used for nucleus accumbens infusion of MN, except that the needles were 12 mm long.

Behavioral Testing

The locomotor activity was measured in wire cages 25×20×36 cm, with 2 horizontal infrared photocell beams located across the long axis of the cage. Beam interruptions were monitored by a microcomputer and printed out every 10 min. Rats were first habituated to the testing cages for 6 hours, prior to the testing day. On the testing day, after 90 min adaptation period, rats were infused with methylnaloxonium or saline 0.9% (control rats) then were given a subcutaneous (SC) injection of heroin (0.5 mg/kg, volume of 1 ml/kg) or saline into the loose skin in the back of the neck. They were immediately returned to the apparatus for 2 hours.

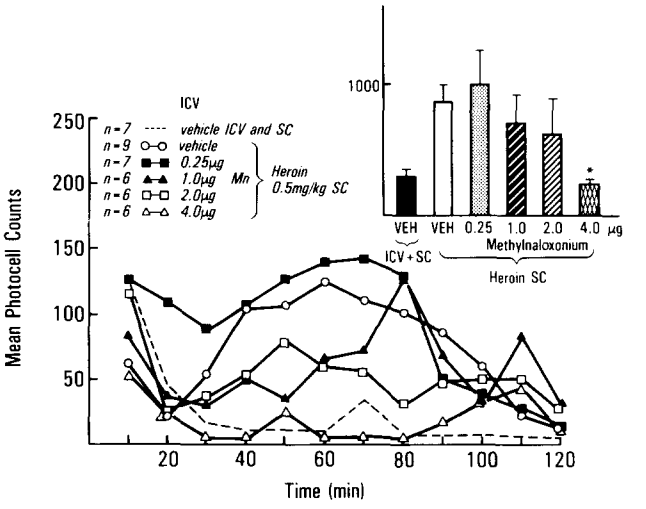

FIG. 1. Mean photocell counts for 2 hours following intraventricular injection of saline or MN at different doses (0.25, 1.0, 2.0, 4.0 μg) and 1 min later, subcutaneous injection of heroin (0.5 mg/kg). Insert: Locomotor activity counts for the total 120 min. *Significantly different from control (heroin SC vehicle) $p<0.05$ by Student's t-test, following overall significant ANOVA, see text.

Histological Analysis

All rats were sacrificed and were perfused intracardially with saline followed by 10% formalin. Samples of eleven rats with cannulae aimed at the VTA and ten rats with cannulae aimed at the N.Acc. all distributed more or less equally across all dose levels were sectioned in a cryostat to verify cannulae locations. Fifty micron sections were cut through the course of the cannula tract and mounted on glass slides. Slides were then examined under a dissecting scope and the tip of the cannula marked on drawings based on König and Klippel [14].

Data Analysis

The number of photocell beam interruptions per 10 min period were subjected to a two factor analysis of variance (ANOVA) with the different doses and saline groups as the independent factors and time as the repeated measure. Because of the regular pattern of activity changes following heroin administration, two subsequent ANOVA's were performed on the data for 0–30 min and 40–120 min after injection. Individual means comparisons were made by using Student's t-test.

RESULTS

The VTA cannulae analyzed histologically were, all except one, localized to a region including and surrounding the VTA that ranged in the AP plane from the posterior part of

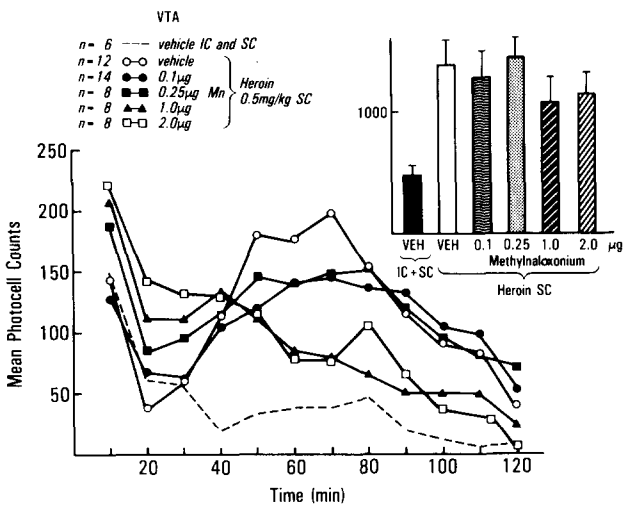

FIG. 2. Locomotor response for 2 hours following bilateral VTA infusion of methylnaloxonium (0.1, 0.25, 1.0, 2.0 μg total dose) followed 1 min later by a subcutaneous injection of heroin (0.5 mg/kg). Insert: Total photocell counts for the 2 hours.

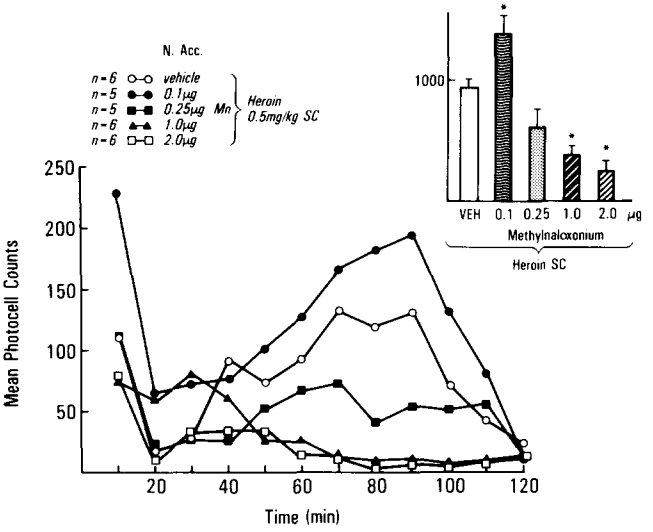

FIG. 3. Locomotor response for 2 hours following bilateral N.Acc.-infusion of methylnaloxonium (0.1, 0.25, 1.0, 2.0 μg total) followed by a subcutaneous injection of heroin (0.5 mg/kg). Insert: Total photocell counts for the 2 hours. *Significantly different from control, $p<0.05$ by Student's t-test.

the medial forebrain bundle where the mammillo tegmental tract joins the fasciculus petroflexus (AP 2420 in König and Klippel atlas [14]). One pair of cannula tips was localized more anteriorly above the medial forebrain bundle at the level of the mammillary bodies (AP 3180 König and Klippel [14]) in the dorsal ventral plane. All cannulae fell below the medial lemniscus and above the interpeduncular nucleus. All cannulae fill symmetrically 0.8–1.2 min lateral to the midline.

The N.Acc. cannulae were all localized within the N.Acc. around the anterior commissure in an anterio-posterior plane ranging from the anterior part of the corpus callosum where the two arms of corpus callosum join into the medial gene of corpus callosum (A9410 according to König and Klippel atlas [14]) to a level just before the beginning of the posterior part of the anterior commissure (AP 8620 König and Klippel [14]). In the dorsal ventral plane all cannulae fell even with or below the anterior commissure. In the lateral medial plane all cannulae fell just medial to the anterior commissure.

Heroin injected subcutaneously at a dose of 0.5 mg/kg elicited a biphasic locomotor response. An initial cataleptic phase (10 to 30 min) was followed by a long-lasting period (40–120 min) of enhanced locomotor activity interrupted by bursts of stereotyped behavior (gnawing, licking, etc.). The activity reached a peak around 1 hour after injection time and lasted up to 2 hours (see Fig. 1).

The intracerebroventricular injection of methylnaloxonium (MN) at doses ranging from 1 μg to 4 μg decreased the locomotor activity for the two hours following the injection, in a dose-dependent way; (ANOVA: $F(4,29)=2.64$, $p<0.05$), t-test comparing 4 μg/2 μl MN plus heroin SC and VEH plus heroin showed a significant difference, $t(13)=3.7$, $p<0.05$, see Fig. 1. Lateral ventricular injections did not significantly reverse the initial depressant effects of heroin (0–30 min), $F(4,29)=2.54$, $p>0.05$, but the highest dose of 4 μg did reverse the subsequent hyperactivity ANOVA: $F(4,29)=2.865$, $p<0.05$; saline vs. 4 μg, $t(13)=4.3$, $p<0.05$.

ANOVA of the MN injection into the VTA (control group: vehicle in the VTA and heroin SC) revealed a significant group × time interaction, $F(44,495)=2.79$, $p<0.05$, but no significant difference over the total 2 hours, $F(49)<1$. Locomotor activity was not significantly higher than the control group (heroin alone) during the first 30 minutes. However, locomotor activity following injection of the highest dose of MN (2.0 μg) into the VTA was significantly increased over control (heroin alone) when measured at the 20 and 30 min time points (Student's t-test: $t(18)=2.8$, $p<0.05$). Some depression of the activity was then observed during 2 hours at the highest doses (1.0 and 2.0 μg), (see Fig. 2).

Analysis of nucleus accumbens data, represented in Fig. 3, revealed a significant main effect of dose, $F(4,23)=15.4$, $p<0.01$, and a group × time interaction, $F(44,253)=2.7$, $p<0.01$. Doses of MN (1.0 and 2.0 μg) that induced only an attenuation of locomotor activity when injected into the VTA completely blocked the heroin-induced activation throughout all of the testing period when injected into the N.Acc. Student's t-test showed a significant difference between doses of MN 1.0 or 2.0 μg (with heroin SC) and control group for total counts (vehicle IC and heroin SC): for 1.0 μg, $t(10)=5.9$, $p<0.01$, and for 2.0 μg, $t(10)=6.7$, $p<0.01$. Also the lowest dose of MN (0.1 μg total dose) resulted in a significant general activation in the first 30 min depressant phase , (ANOVA: $F(4,23)=5.85$, $p<0.05$; saline vs. 0.10 μg, $t(9)=3.4$, $p<0.05$), similar to the tendency observed for 0.25 μg injected into the ventricle. A dose as low as 0.25 μg MN significantly reversed the subsequent hyperactivity (40–120 min), $t(9)=2.1$, $p<0.06$.

DISCUSSION

The data presented in this study support the notion that both the N.Acc. and VTA mediate some aspects of heroin-induced locomotor activation in rats but probably by different mechanisms. Previous studies showed that injection of

opiates in the ventral tegmental area results in an increase of locomotor activity and a general behavioral activation [9]. Microinjections of an enkephalin analog, D.ala.Met-enkephalinamide (DALA) into the VTA [4, 10, 11, 12] elicited a potent, long-lasting, dose dependent and opiate-specific behavioral activation. A pretreatment with naloxone, an opiate receptor antagonist, resulted in a blockade of this activation [4, 10, 12]. Similar effects have been seen with infusion of endorphins in the same area [21]. This VTA activation is likely to be due to an increased release of DA in the mesolimbic areas, since the VTA response to DALA was antagonized by a dopaminergic receptor antagonist (α-flupenthixol) [10], or by destruction of the mesolimbic DA system with 6OHDA [11,21].

However, Pert and Sivit [15] also have reported that microinjections of morphine into the nucleus accumbens resulted in an increase in spontaneous motor activity in rats. A significant elevation in locomotion and rearing preceded by a cataleptic phase, depending on the dose injected, was also seen after DALA microinjection into the N.Acc. [8, 11, 15]. These effects were reversed by a pretreatment of naloxone. However, in contrast to the activation following opiate injected into the VTA, this effect is not antagonized by haloperidol (a dopaminergic antagonist) [15], nor by 6OHDA induced depletion of DA in the accumbens [11] suggesting that opiates increase motility in this region by a nondopaminergic action.

However, an important involvement of the N.Acc. in the opiate-induced activation is suggested by our results. The blockade of heroin-induced activation is more dramatic, when MN is injected into the N.Acc. Furthermore, lower doses are required to attenuate the locomotor stimulation and in a recent study [5], electrolytic lesion of the VTA seem less effective in attenuating a morphine-induced locomotor hyperactivity than a N.Acc. lesion.

The fact that intracerebral injection of methylnaloxonium is significantly less effective in blocking heroin-induced activation in the VTA than in N.Acc. suggests that the N.Acc. may be the critical site for the excitatory effects of opiates. The differences observed with other studies, in which stimulation of the mesolimbic dopaminergic pathway by opiates appears to be very potent, may be due to the fact that in those studies opiates are injected intracerebrally directly into the site of action. Other differences may result from the fact that the opioid peptide most frequently used, D.ala. enkephalin, binds preferentially with a delta type opiate receptor [20], which is found in high concentration in the VTA. In contrast, heroin is degraded into morphine into the brain [22], and thus presumably acts as a ligand for the mu type opiate receptor [6,17].

The behavioral activation observed following stimulation of the N.Acc. opiate receptors may be postsynaptic and indepedent of the mesolimbic system since lesions or pharmacological blockade of the mesolimbic DA projection do not block the locomotor activation derived from direct intra-N.Acc. opioid peptide administration [11].

In addition, lesions of the mesolimbic DA system fail to alter heroin induced locomotor activation (Vaccarino, Amalric, Swerdlow and Koob, unpublished results). In fact, Pollard *et al.* [18] report that only 50–70% of the opiate receptors (as measured by tritiated naloxone binding) are localized on dopaminergic neurons.

In summary our findings indicate that heroin, at least in the rat, produces excitatory effects by activating to some extent the DA mesolimbic pathway, but more importantly, by stimulating a postsynaptic pathway in the nucleus accumbens which is independent of a dopaminergic transmission. What pathways, receptors, and endogenous ligands are involved remains to be determined.

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