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Morphine-Dopamine Interaction: Ventral Tegmental Morphine Increases Nucleus Accumbens Dopamine Release

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LEONE, P., D. POCOCK AND R. A. WISE. *Morphine-dopamine interaction: Ventral tegmental morphine increases nucleus accumbens dopamine release.* PHARMACOL BIOCHEM BEHAV **39**(2) 469–472, 1991.—Microdialysis and high-performance liquid chromatography with electrochemical detection were used to determine extracellular levels of dopamine following ventral tegmental morphine injections into chloral hydrate-anesthetized rats. Morphine (13.2 nanomoles in 0.5 μl of Ringer's solution) caused 50–150% increases in nucleus accumbens dopamine and metabolites; latency for the effect was on the order of 15 min with peak effects occurring in 30–50 min. Contralateral dopamine levels were influenced only minimally. These data suggest opiate receptors in or near the ventral tegmental area as sites of the opioid action that is responsible for opioid activation of the mesolimbic dopamine system.

Morphine Ventral tegmental area Dopamine Nucleus accumbens Opioids

SYSTEMIC morphine injections increase the firing of ventral tegmental dopamine neurons (15,18) and thus increase the release of dopamine from nucleus accumbens terminals of the meso-limbic dopamine system (4,5). The dopaminergic neurons appear to be disinhibited, rather than directly activated by local morphine, and the disinhibition may involve long-axon inputs to the region of the cell bodies, since morphine does not accelerate dopaminergic neurons in culture (28) or in tissue slice preparations (24). The actions of morphine on the mesolimbic dopamine system and on its afferents in nucleus accumbens are thought to account for several behavioral effects of opiates, including locomotor effects (11,12), facilitation of feeding (8, 10, 16) and brain stimulation reward (3,9), and the direct habit-forming effects of morphine as demonstrated in lever-pressing (1, 27, 29) and place-preference (2, 19, 26) experiments.

The fact that morphine does not activate dopaminergic neurons in culture or tissue slices suggests that the activation is indirect and involves, in some way, long-axon afferents to the dopaminergic cells (24). It is not known to what degree ventral tegmental actions contribute to the increases in nucleus accumbens dopamine that are induced by systemic opiate injections, however. Morphine is known to have more than one effect on nucleus accumbens dopamine levels; nucleus accumbens morphine attenuates the dopamine release induced by systemic morphine (22). Moreover, morphine could influence nondopaminergic afferents to nucleus accumbens—afferents that can influence dopamine release independent of dopaminergic impulse flow (13,17).

One approach to identifying morphine actions triggered in the region of the dopamine cell bodies is to study the effects of injecting low doses of morphine directly into the ventral tegmental area. Such injections, like systemic morphine injections, increase locomotion (11) and feeding (8, 10, 16), potentiate the rewarding effects of hypothalamic (3,9) and central gray (23) electrical stimulation, and are rewarding in the place-preference (2,19) and self-administration (1, 27, 29) paradigms. Each of these actions is presumed to be mediated by the effects of ventral tegmental morphine on DA impulse flow. However, direct measurements of nucleus accumbens dopamine release (4) have not yet been made in animals receiving microinjections of morphine into the ventral tegmental area. The purpose of the present investigation was to determine if morphine injections into the VTA cause dopamine release similar to that resulting from systemic application of the drug.

METHOD

Animals and Surgery

Nine male Long-Evans rats weighing 375–400 g were used. They were housed in a 14-h light, 10-h dark cycle and tested during the light phase. Under pentobarbital (65 mg/kg, IP) each was implanted with bilateral 20 ga guide cannulae above the NAS and with a unilateral 22 ga guide cannula above the VTA. With the incisor bar 5 mm dorsal to the interaural line, stereotaxic coordinates were 3.2 mm anterior to bregma, 2.6 mm lat-

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eral (but angled toward the midline at 10° from the vertical), and 5.1 mm ventral to the skull surface (NAS) and 3.0 mm posterior to bregma, 2.9 mm lateral (angled at 10°, as above), and 7.6 mm ventral to the skull surface (VTA). Cannulae were anchored to each other and to four skull screws with acrylic dental cement. Obturators extending 4 (NAS) or 1 (VTA) mm ventral to each guide cannula were put in place at the time of surgery and removed at the time of testing.

Microdialysis Probes

Removeable microdialysis probes were constructed after the method of Robinson and Whishaw (21). The probes consisted of a 26 ga stainless steel outer cannula, cemented to a 4 mm length of cellulose dialysis fiber (Brain Research Institute, i.d. = 152 μm; o.d. = 406 μm; pore diameter sufficient for 6,000 mol. wt. particles) which was plugged with acrylic cement; the return was through an inner cannula of silica capillary tubing (Polymicro Technologies, o.d. = 150 μm; i.d. = 75 μm) which terminated 1 mm from the plugged tip of the dialysis tubing. The silica tubing exited the polyethylene tubing through a hole that was sealed with epoxy cement, terminating in a collection vial. The probes were stored in a covered dry reservoir. A few hours before use they were dipped first in a beaker of 50% ethanol and then in a beaker of Ringer's solution and flushed with Ringer's solution (20 minutes in each condition). Probe recovery was determined at room temperature in Ringer's solution containing 50 pg/μl of dopamine and 1000 pg/μl of DOPAC, HIAA, and HVA; recovery of dopamine was consistently 8–10%.

Microdialysis Procedure

On test days the animals were anesthetized with chloral hydrate (400 mg/kg, IP) and injected with atropine sulphate (0.5 mg/kg, IP) to counteract salivation and mucous secretions; supplemental doses (200 mg/kg) of chloral hydrate were given when signs of limb movement were seen. Microdialysis probes were inserted bilaterally into NAS and were perfused with sterile Ringer's solution at a flow rate of 2 μl/min. Samples taken for the first hour were discarded as potentially contaminated by injury-induced dopamine release; preliminary observations indicated no detectable dopamine release (our system is sensitive to 2 pg/40 μl sample) when the animals were perfused with Ca^{+}-free Ringer's solution, suggesting that insertion of the probes through the previously implanted guide cannulae did not cause any long-lasting disturbance in baseline dopamine release. Dialysate samples were collected at 23-min intervals and either injected immediately onto the chromatography column or frozen in dry ice and stored at −80°C for 6 hours before being quickly thawed and injected into the column. Alternating samples were injected or frozen, such that fresh samples and frozen samples were counterbalanced across sample times and across animals. Baseline samples were collected until dopamine peaks were stable, and the animals were then given morphine infusions into the VTA (13.2 nM/0.5 μl over 1.5 min). Dialysate samples were collected for another 4 hours, and then the animal was transcardially perfused with saline followed by 10% formalin. The brain was removed immediately, stored in formalin for one or more days, frozen, and sliced in 40 micron sections for localization of cannula tracks.

Analytical Procedure

Dialysate was assayed for dopamine using isocratic, reverse-phase high performance liquid chromatography with electrochemical detection. For each sample, 40 μl of dialysate was injected into a 15 cm × 4.6 mm (i.d.) column (Supelcosil LD-18-DB, 5 micron, Supelco, Bellefonte, PA) via a Rheodyne injection valve with a 100 μl sample loop. The recycled mobile phase (3.5% acetonitrile, 0.008% sodium octyl sulfate, and 0.010% EDTA in 27.8 mM sodium acetate with pH adjusted to 3.8 with glacial acetic acid) was pumped through a pulse dampener (SSI Lo Pulse Model LP-21, State College PA) at 1.4 ml/min with a Model 510 Waters pump. DA eluted in approximately 9 minutes and was clear of DOPAC, HIAAA, and HVA (6, 12, and 18 min, respectively). The detector (ESA Model 5100A Coulometric System, Chromatographic Sciences Co., Montreal) contained, in series, a conditioning electrode (Model 5021 Conditioning Cell) set at +0.45 V and two analytical electrodes (Analytical Cell Model 5011), the first set at +0.05 V and the second at −0.35 V. The last electrode was connected to a Waters Data Module (Model 742B); DA was quantified as it was reduced, having first been oxidized completely. The integrator was calibrated, using peak area, at the beginning of each series of experiments, with a solution of fresh DA (20 pg/40 μl), DOPAC (800 pg/40 μl), HIAA (400 pg/40 μl), and HVA (800 pg/40 μl) standards (Sigma) in Ringer's solution; calibration was checked periodically with these and double these standards.

RESULTS AND DISCUSSION

VTA morphine increased NAS dopamine concentrations within 15 min of injection (Fig. 1); the effects lasted on the order of three hours. Analysis of variance revealed a significant main effect of drug, $F(1,12) = 12.2$, $p<0.005$, and time, $F(5,60) = 2.28$, $p<0.05$. The drug effect was marked only on the side ipsilateral to the drug injection, but there was some evidence of a contralateral effect and the drug-side interaction only approached statistical significance, $F(1,12) = 3.6$, $p<0.082$. The dopamine recovery from the frozen samples was unsatisfactory [Drug ×

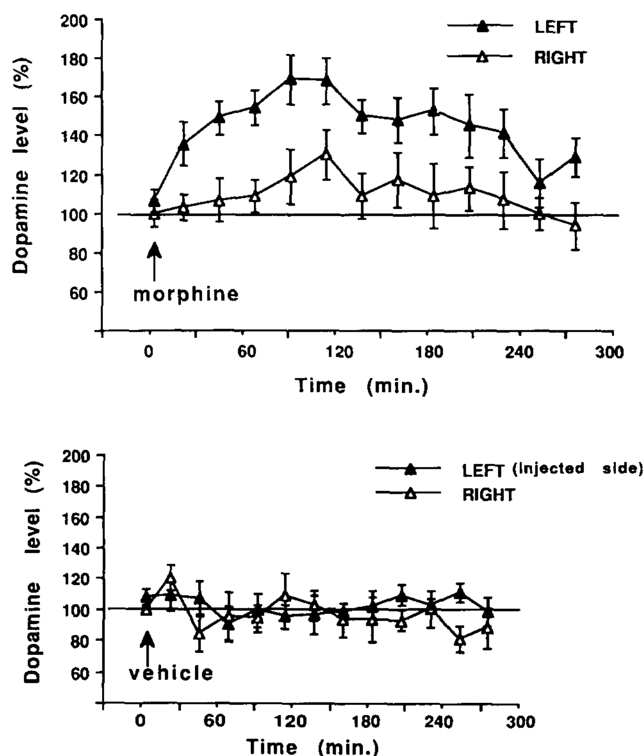

FIG. 1. Dopamine levels in nucleus accumbens perfusates following ventral tegmental injections of morphine (top) or vehicle (bottom).

Temp interaction: $F(1,12)=11.4$, $p<0.006$]; recovery was particularly poor from the frozen samples contralateral to the drug injections (where the drug-induced dopamine release was minimal).

The present data confirm that actions of morphine in or near the ventral tegmental area cause increased release of dopamine in the NAS. The mechanism of action presumably involves the known ability of these injections to increase impulse flow in the mesolimbic dopamine system (15,18) and presumably results from disinhibition rather than direct excitation of the dopamine cells (24). Current evidence suggests that any actions that morphine has in the region of dopamine nerve terminals act to antagonize the effects that are triggered at the dopamine cell body, acting perhaps on opioid receptors on dopamine nerve terminals in such a way as to decrease impulse-dependent dopamine release (22,31).

The loss of DA from frozen dialysate is unfortunate, since it would ease the problems of equipment shortage if frozen samples could be analyzed off-line. With refinements of method it may be possible to improve recovery from frozen samples; simple use of frozen samples is not recommended, however, until such refinements are made. Even in the present case where frozen samples were counter-balanced and averaged across experimental and control conditions, the frozen samples added significantly to the error variance and tended to obscure the time course of the morphine effects.

Despite anesthesia, which generally decreases dopaminergic activation (5,15), strong ipsilateral increases in NAS dopamine were observed in the present experiment. Some indication of contralateral increase was also noted; contralateral release was not unexpected, since there is a weak contralateral projection of the nigro-striatal dopamine system (20) and presumably of this system as well. Stronger contralateral responses might have been seen in unanesthetized animals, but the contralateral response was weak in comparison to the ipsilateral response.

The fact that dopamine levels increased despite surgical anesthesia makes it clear that dopaminergic activation is not a simple consequence of motor activity. Rather, dopamine—particularly meso-limbic dopamine—seems involved in some more subtle aspect of sensory-motor integration. The locomotion induced by activating the dopamine system with ventral tegmental morphine is under considerable environmental control (7,30), and even the stereotyped behaviors induced by high doses of amphetamine are largely sense dominated (6,25).

The effects of ventral tegmental morphine were not challenged with naloxone in the present study in order to follow the full time course of the morphine effects. The behavioral effects (7–9) of these doses of ventral tegmental morphine are, however, known to be reversed by naloxone at doses of 1 and 2 mg/kg IP. Thus the present data suggest that morphine exerts a pharmacological action in or near the ventral tegmental area, and that this action results in disinhibition of dopaminergic cell firing and consequent release of dopamine in the nucleus accumbens.

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