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Contraversive circling induced by ventral tegmental microinjections of moderate doses of morphine and [D-Pen^2,D-Pen^5]enkephalin

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Unilateral ventral tegmental area (VTA) injections of morphine and [D-Pen^2,D-Pen^5]enkephalin (DPDPE), caused contraversive circling at doses of 1.2, 12, and 24 nmol. Similar doses of the selective κ-agonist U-50,488H were ineffective. These data suggest a common mechanism for the circling, locomotion and facilitation of brain stimulation reward caused by VTA morphine, and distinguish this mechanism for that of feeding which is caused by both morphine and κ-actions in this region.

Injections of morphine into the ventral tegmental area have a variety of behavioral effects. They stimulate locomotion[11], facilitate feeding[8,10] and brain stimulation reward[4,9], and have direct rewarding actions in their own right[3,17]. It is important to determine which of these behaviors are homologous, deriving from a common brain action and mechanism, and which are independent, deriving from activation of distinct or partially distinct circuitry[19]. The effects of morphine on stimulation-induced feeding are mimicked by both [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and U-50,488H[10], but the effects on brain stimulation reward are mimicked by DPDPE but not U-50,488H[9]. Inasmuch as forward locomotion has been argued to be a common denominator of the drive-like and reward-like effects of lateral hypothalamic stimulation[5,19], and inasmuch as the effects of U-50,488H dissociate these two effects of stimulation, it is of interest to determine if ventral tegmental injections of U-50,488H mimick or fail to mimick ventral tegmental injections of morphine.

The ventral tegmental effects of morphine have been studied in two ways. Bilateral injections have been studied in activity boxes, and unilateral injections have been studied in rotation apparatus. Bilateral injections of morphine[11,12] increase locomotor activity, and unilateral injections cause asymmetrical locomotion[6,7] in an enclosed[20] environment. In the present experiment the effects of the selective δ-agonist, DPDPE[15], and the selective κ-agonist, U-50,488H[18], were compared to the effects of morphine.

Twenty male Long–Evans rats (400 g), housed individually with free access to food and water, were maintained on a normal 12-h light, 12-h dark cycle. Each was implanted (under pentobarbital anesthesia, 60 mg/kg, i.p.) with a 24-gauge guide cannula which terminated 1 mm above the ventral tegmental area. The cannulae approached the target at a 15% angle from the vertical in order to bypass the periaqueductal gray where drug reflux might have actions that would be incompatable with rotation[1,2]. Pellegrino et al.[16] stereotaxic coordinates for the intended injection site (1 mm beyond the tip of the guide cannula) were 3.6 mm posterior to bregma, 0.7 mm lateral, and 8.6 mm ventral to the surface of the dura. Stainless-steel blockers were kept in the guide cannulae between injections of drug.

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Each animal was tested once under each of 3 doses (1.2, 12, and 25 nmol) of each drug; the injections were given in a counterbalanced order at 3-day intervals. The range of selected doses was chosen to span the range of effective doses in feeding[10] and self-stimulation[9] studies.

Injections were made with a 1-μl Hamilton microsyringe connected by polyethylene tubing to a 31-gauge injection cannula which penetrated 1 mm beyond the tip of the guide cannula. Doses of 1.2, 12, and 25 nmol were injected in 0.4 μl over 30 s; injection cannulae were left in place for 2 min after each injection. The injectors and tubing used to inject DPDPE were coated with bovine serum albumin, in order to minimize unwanted binding of peptide to these surfaces.

Following initial testing, the 12-nmol dose was challenged with 2 mg/kg of naloxone given i.p. In each case, near-maximal levels of circling were established before naloxone was given. Naloxone was given 30 min after morphine and 10 min after DPDPE.

Behavioral testing was carried out in 28-cm diameter enclosures with a flat floor. A freely turning cable was connected to the cannula assembly on the head of each animal; the cable was connected to a rotometer that counted full 360° rotations in each direction. Counts were recorded by microprocessor at 5-min intervals for 30 min before and 120 min after each injection. Data were analyzed by means of the Wilcoxon signed-ranks test.

Following the last test the animals were sacrificed under chloral hydrate anesthesia (400 mg/kg). They were perfused with 0.9% saline followed by 10% formalin. Frozen sections (40 μm) were stained with thionin and examined to determine the location of each cannula tip.

Each tested dose of morphine and DPDPE increased contraversive circling; there was no increase in ipsiversive circling with either agent (Fig. 1). U-50,488H had no comparable effects. Morphine caused the longest-lasting effects; even the lowest dose was still effective 2 h after injection (Wilcoxon $T = 0, n = 0, P < 0.01$). The peak values reached at the two high doses did not differ statistically ($T = 81.5, n = 19, P > 0.5$). In fact, when considered over the duration of the experiment, the 12-nmol morphine injections appeared more effective, on average, than the 25-nmol injections ($T = 85.0, n = 29, P < 0.01$).

DPDPE caused graded effects (Fig. 1), but the durations were much shorter than those for morphine. The low dose was no longer different from saline by 60 min after injection ($T = 8.5, n = 9, P > 0.05$). The effects of 12 and 25 nmol were no longer significantly different from saline by 90 min after injection ($T = 21, n = 13, P > 0.05$, in each case). The 3 doses caused peak effects within 10 or 15 min of injection; peak effects for 12 and 25 nmol did not differ statistically ($T = 57, n = 17, P > 0.05$), but the average effect over the duration of the session was greater in the case of the higher dose ($T = 27, n = 30, P < 0.01$).

The effect of U-50,488H was not different from saline at the 1.2 and 12 nmol doses ($T = 151, n = 30, P > 0.05$, and $T = 129, n = 29, P > 0.05$, respectively). The dose of 25 nmol appeared to induce a small and very short-lived effect, but it was not statistically reliable over the period tested.

The effects of morphine and DPDPE were reversed by naloxone (Fig. 2). Microinjections of 12 nmol of morphine and DPDPE were followed by naloxone administered 30 min and 10 min later, respectively. Morphine-induced circling was no different than control within 15 min ($T = 7.5, n = 7, P > 0.05$) and DPDPE-induced circling was no different than control within 20 min ($T = 31, n = 10, P > 0.05$). In the case of morphine, the effectiveness of naloxone was incomplete after 45 min when weak but significant morphine-induced contraversive circling again emerged ($T = 0, n = 6, P < 0.05$). A few control animals have been tested with intracranial injections of morphine followed by i.p. saline injections; these i.p. saline injections did not significantly affect the rotational behavior induced by the intracranial infusions (data not shown).

Cannula tips were all in the ventral tegmental area. In general, the strongest opioid-induced circling was seen in the animals with cannula tips located within an area bounded by the substantia nigra, the interpeduncular nucleus, and the medial lemniscus. Each animal that showed high sensitivity to morphine also showed high sensitivity to DPDPE.

The relatively equal potency of morphine and DPDPE and the marked lack of effectiveness of U-50,488H suggest that contraversive rotation produced by ventral tegmental opioid injections is me-

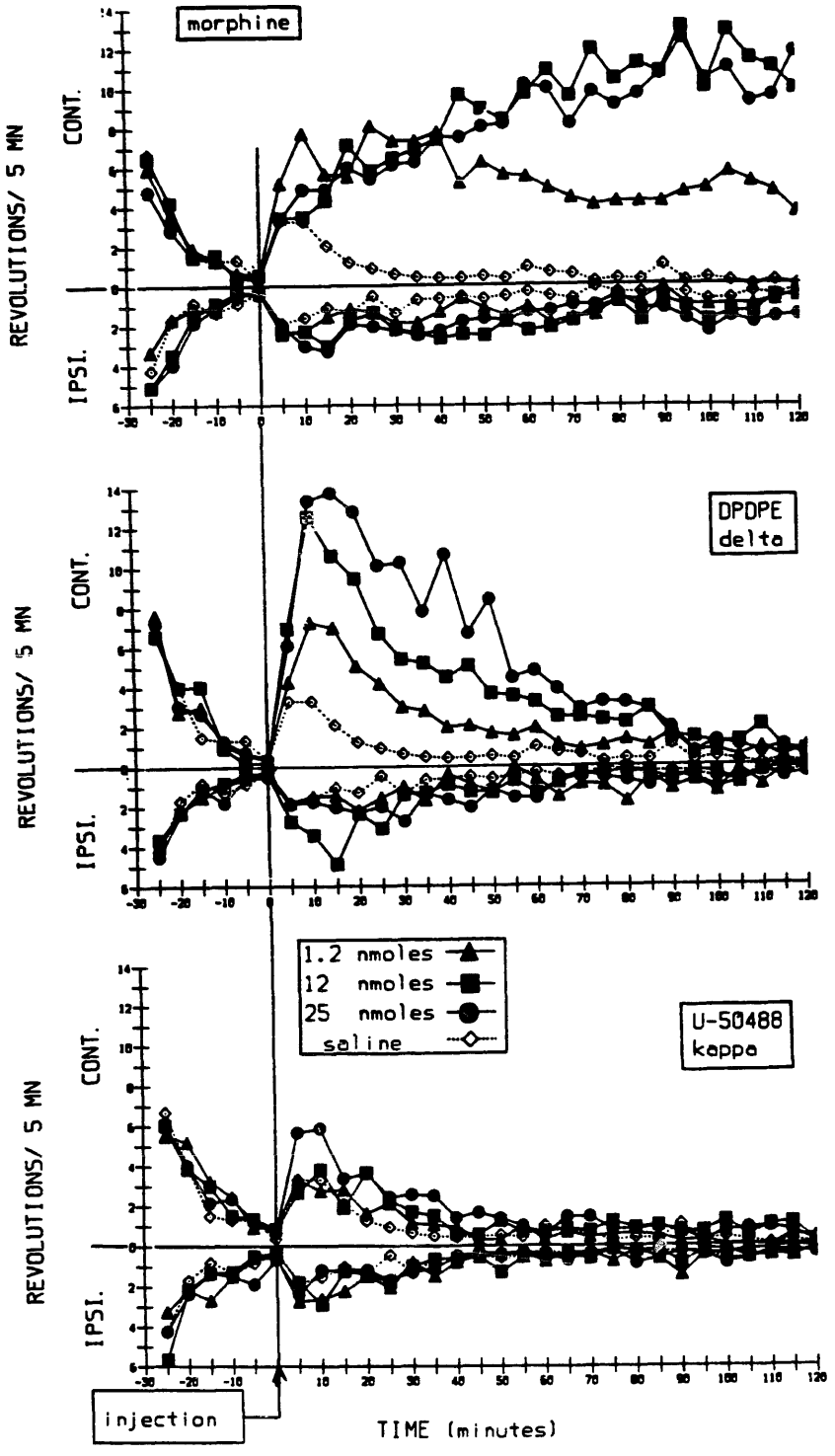

Fig. 1. Rotation induced by ventral tegmental microinjection of morphine, DPDPE, and U-50,488H. Twenty rats were tested in each condition: representative error bars are shown for the middle doses in Fig. 2.

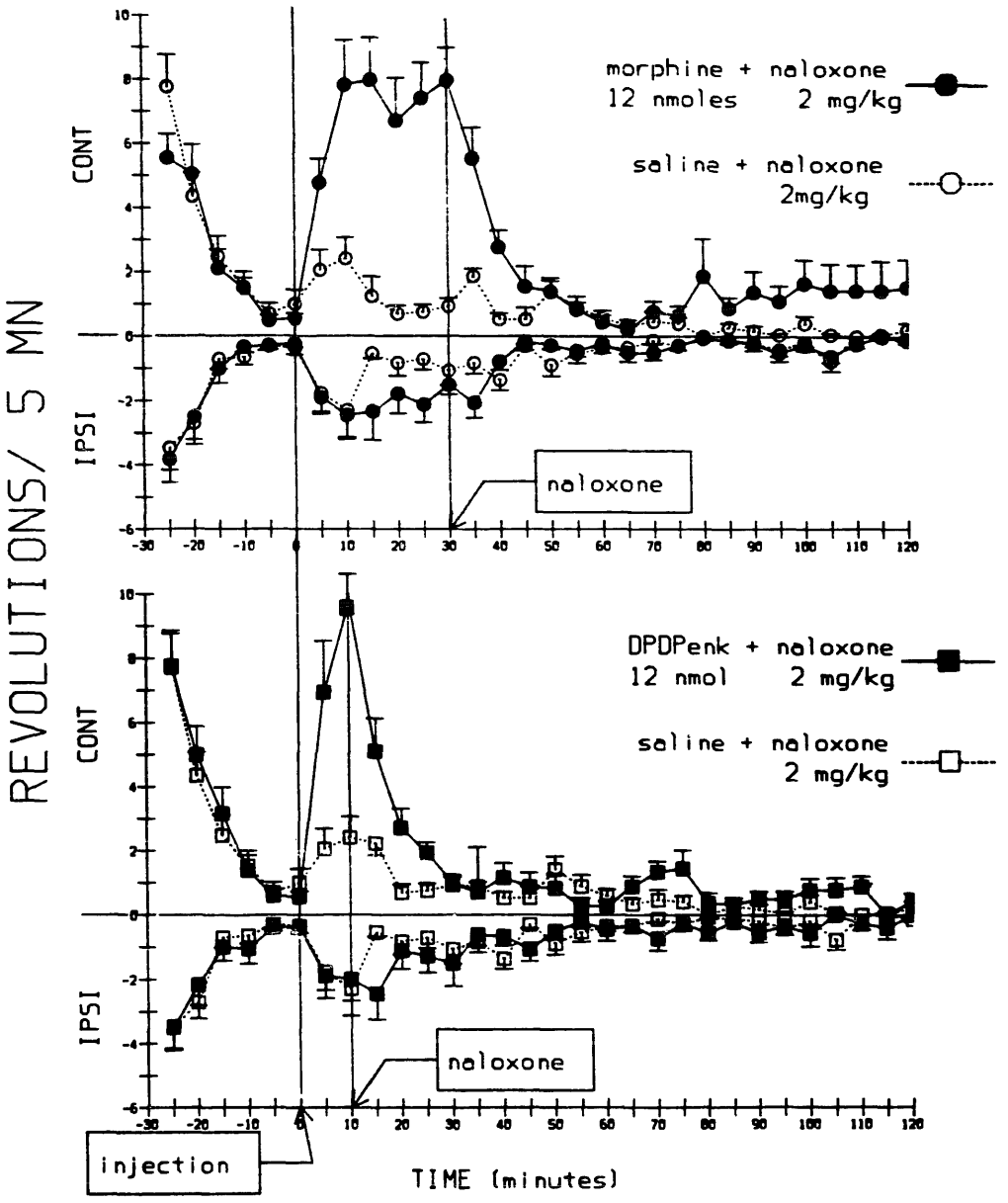

Fig. 2. Effects of naloxone (2 mg/kg, i.p.) on morphine- and DPDPE-induced rotation.

diated by δ-receptors or by μ- and δ-receptors. Morphine and DPDPE had similar peak effects at each dose tested, though the morphine reached peak effect much more slowly and had much longer action than DPDPE. The level of circling produced by 25 nmol of U-50,488H (after subtracting the non-specific activity which is reflected in saline data and in ipsilateral turning data) was minimal compared to even the 1.2 nmol doses of morphine and DPDPE. The locomotor effects of bilateral ventral tegmental opioids have been argued to be mediated by activation of μ-receptors[13], and it seems clear[7] that the contraversive circling induced by unilateral injections involves the same anatomical substrate as the locomotion induced by bilateral injections.

These data are important because they make it

clear that the locomotor-stimulating effects of ventral tegmental opioids, like their reward-facilitating effects[9] are not homologous with the facilitation of feeding that can be induced equally by ventral tegmental morphine, DPDPE, and U-50,488H[10]. This falsifies a significant portion of the hypothesis of Glickman and Schiff[5], recently extended by Wise and Bozarth[19], that 3 classes of behavior that are induced by lateral hypothalamic electrical stimulation — forward locomotion, brain stimulation reward, and stimulation-induced feeding — are homologous, arising from activation of a common medial forebrain bundle substrate. The possibility remains that medial forebrain bundle forward locomotion and brain stimulation reward arise from activation of a common mechanism, but it would appear that the mechanism of stimulation-induced feeding[10] is, at least to some extent, distinct from that of forward locomotion and self-stimulation. Since ventral tegmental opiates activate ventral tegmental dopaminergic neurons, and since the mechanisms of forward locomotion, brain stimulation reward, and stimulation-induced feeding all appear to depend importantly on this activation[19], one possibility for further consideration is that different subsets of dopaminergic neurons are involved in feeding effects on the one hand and locomotor and reward-related effects on the other.

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