Pharmacology Biochemistry & Behavior, Vol. 30, pp. 1077–1079. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

RAPID COMMUNICATION

Potentiation of Morphine-Elicited Circling by Dopaminergic Uptake Blockade

ALAIN GRATTON,*[1] BARRY J. HOFFER,† GREG A. GERHARDT†‡ AND ROY A. WISE§

**Douglas Hospital Research Center, McGill University, Verdun, Quebec, Canada H4H 1R3*
and Departments of †Pharmacology and ‡Psychiatry
University of Colorado Health Sciences Center, Denver, CO 80262
and §Center for Studies in Behavioral Neurobiology, Department of Psychology
Concordia University, Montreal, Quebec, Canada, H3G 1M8

Received 23 May 1988

GRATTON, A., B. J. HOFFER, G. A. GERHARDT AND R. A. WISE. *Potentiation of morphine-elicited circling by dopaminergic uptake blockade.* PHARMACOL BIOCHEM BEHAV **30**(4) 1077–1079, 1988.—Contraversive circling induced by unilateral infusions of morphine (7.5 nanomoles or 5 μg) into the ventral tegmental area was studied under conditions of dopamine uptake inhibition. Dopamine uptake blockade with either nomifensine (2.5 mg/kg) or GBR 13069 (2.0 mg/kg) resulted in a 3-fold increase in the rate of circling. Naloxone (1.5 mg/kg) attenuated but did not completely block the potentiated circling. These data are consistent with the hypothesis that morphine causes contraversive circling through local activation or disinhibition of dopaminergic cell firing.

Morphine Dopamine Circling Nomifensine GBR-13069

LOCAL injection of morphine into the ventral tegmental area (VTA) has a variety of interesting behavioral actions. It facilitates feeding (9), sexual behavior (J. Mitchell, unpublished Ph.D. thesis, Concordia University), and forward locomotion (11). It potentiates the rewarding effects of hypothalamic (3,10) and pontine (16) brain stimulation, and it is rewarding in its own right (1, 2, 15).

Each of these behavioral actions is thought to be mediated by activation or disinhibition of local, VTA, dopaminergic mechanisms; opiates increase dopaminergic cell firing (13,14) and nucleus accumbens dopamine turnover (7). Challenge of these behaviors with dopamine receptor blockers, however, does not provide a convincing test of the dopaminergic hypothesis, since dopamine antagonists cause a general attenuation of behavior.

The stimulation of locomotion by unilateral ventral tegmental morphine results in circling which is usually [but not necessarily: Wise and Holmes (17)] contraversive (6,8). This circling is blocked by doses of dopamine antagonists that do not block muscimol-induced circling (6). Inasmuch as there are many ways that dopamine blockade could interfere with behavior, it is of interest to determine the effects on morphine-elicited circling of drugs which enhance impulse-dependent dopaminergic actions. The present study was designed to assess the effects of the dopamine uptake blockers nomifensine and GBR-13069.

METHOD

Under sodium pentobarbital anesthesia, male Sprague-Dawley rats were stereotaxically implanted with unilateral, chronic stainless steel guide cannulae aimed at a point 1 mm above the VTA (A-P=5.3 mm behind bregma, M-L=0.6 mm from the midline, D-V=8.0 below dura). The cannulae were angled 10° off the vertical plane to prevent efflux of the drug solution into the opiate receptor field of the periaqueductal gray. The animals were also fitted with plastic head connector onto which a flexible lead could be attached. The cannula and connector were held together and anchored to the skull by acrylic dental cement applied around screws embedded in the cranium. Blockers, made of stainless steel wire, were inserted into the guide cannulae between testing sessions.

The morphine sulfate solution (7.5 nanomoles; 5 μg prepared in normal saline) was microinjected into the VTA in

[1]Requests for reprints should be addressed to Alain Gratton, Ph.D., McGill University, Douglas Hospital Research Center, 6875 Boulevard LaSalle, Verdun, Quebec, Canada H4H 1R3.

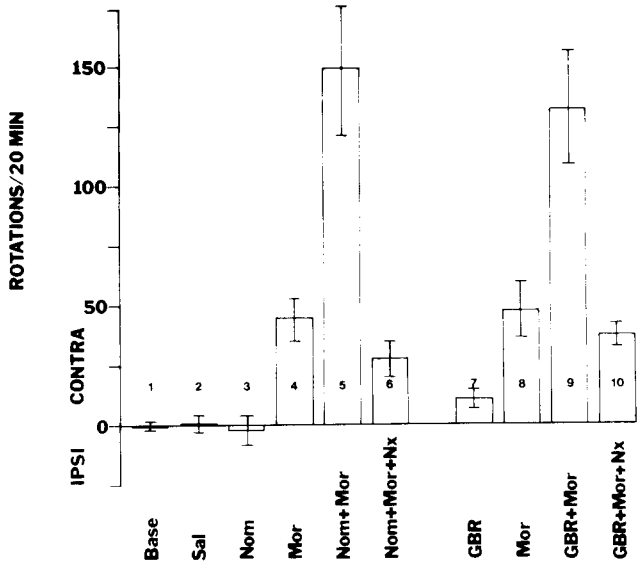

FIG. 1. Rate of circling following various drug treatments. Bars show average rate of circling ±S.E.M. The average rate of circling during baseline (Base) condition or following subcutaneous administration of saline (Sal), nomifensine (Nom) or GBR-13069 (GBR) are represented by bars 1, 2, 3 and 7, respectively. The average rate of circling during the last hour of the session (60–120 minutes following intra-VTA morphine) is represented by bars 4 and 8 for the nomifensine and GBR-13069 groups, respectively. Bars 5 and 9 show respectively the nomifensine- and GBR-13069-potentiated average rate of circling for the last hour of the session. Bars 6 and 10 are the average data obtained during the last hour of testing when the animals were treated with naloxone (Nx).

a volume of 0.5 μl over a period of 1 minute. The microinjections were performed by inserting, into the guide cannula, an injection cannula that protruded 1 mm beyond the tip of the guide cannula into the VTA and that was connected to a 1.0 μl microsyringe by way of a polyethylene tube. The injection cannula was left in the guide cannula 1–2 minutes following the injection.

The animals were tested for rotation in a 35 cm diameter cylinder. The plastic head connector was attached to a bidirectional swivel by means of a flexible lead. Quantification of the direction and rate of circling involved letting a spool of thread wind around the animal's lead and marking off the elapsed time at 20 minute intervals with a piece of masking tape. This procedure has been previously shown to provide reliable measurements of rotation induced by intra-VTA morphine (6).

A testing session lasted 200 minutes. Baseline data were obtained during the first 40 minutes. The animals then received, on different days, a subcutaneous injection of either nomifensine (2.5 mg/kg), GBR-13069 (2.0 mg/kg) or normal saline followed 40 minutes later by an intra-VTA infusion of morphine. Sixty minutes after the morphine injection, animals were given either naloxone (1.5 mg/kg, IP) or normal saline as a control and the rate of rotation was measured for a final 60 minute period. At least one day separated each of the 3 drug tests.

At the end of the experiment the animals were deeply anesthetized with chloral hydrate (400 mg/kg, IP) and transcardially perfused with saline followed by a 10% formalin solution. The brains were then frozen and sliced in 40 μm sections for localization of the guide cannula track.

The magnitude of the potentiation of circling produced by nomifensine and GBR-13069 as well as its subsequent inhibition by naloxone were statistically tested with correlated Student's *t*-tests corrected for the number of comparisons performed. The correction called for an alpha level of 0.0125.

RESULTS AND DISCUSSION

As has been previously established, unilateral infusions of morphine into the VTA elicited contraversive circling (see Fig. 1). The onset of circling occurred within 20 minutes after the injection of morphine and peak rates of 30 to 50 rotations/20 minutes were achieved 60 to 80 minutes postinjection. Pretreating the animals with either nomifensine (n=5) or GBR-13069 (n=3) resulted in significantly higher rates of morphine-elicited circling than was seen with morphine alone [nomifensine: $t(4)=5.079$; GBR-13069: $t(2)=7.582$]. Neither nomifensine or GBR-13069 alone produced consistent circling and the two drugs potentiated morphine-elicited circling to approximately the same extent. The increased rate of circling resulting from uptake blockade was proportional to the rate of circling elicited by morphine alone. In other words, the factor by which the rate of circling was augmented following uptake blockade was a constant (approximately 3-fold). Finally, the rate of both nomifensine- and GBR-13069-potentiated circling was significantly attenuated by naloxone [nomifensine: $t(4)=6.879$; GBR-13069: $t(2)=9.484$].

Potentiation of circling elicited by intranigral morphine has been previously shown following amphetamine administration (9). The present data indicate that the catecholamine uptake blocker, nomifensine (5) and the selective DA uptake blocker GBR-13069 (4), also potentiate circling elicited by intra-VTA infusions of morphine. The simplest interpretation of the present data is that intra-VTA morphine elicits contraversive circling by activating or disinhibiting dopaminergic cell activity and that nomifensine and GBR-13069 potentiated circling by increasing the synaptic concentration of DA in the forebrain. That the circling elicited by morphine is mediated by an opiate receptor is suggested by the fact that naloxone significantly attenuated the behavior. The present data are consistent with the notion that the locomotor effects of opiates are due to an interaction with dopaminergic cell bodies of the ventral tegmentum. The present data also indirectly support the idea that the positive reinforcing properties of opiates, like those of psychomotor stimulants, are mediated by ventral tegmental DA neurons.

ACKNOWLEDGEMENTS

Supported by NSERC of Canada and by USPHS grants DA02429, NS09199 and AG06434. Special thanks to Dr. Richard Heikkila for generously providing the GBR-13069.

REFERENCES

1. Bozarth, M. A. Opiate reward mechanisms mapped by intracranial self-administration. In: Smith, J. E.; Lane, J. D., eds. The neurobiology of opiate reward processes. New York: Elsevier; 1983:331–359.
2. Bozarth, M. A.; Wise, R. A. Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sci. 28:551–555; 1981.
3. Broekkamp, C. L. E.; Van den Bogaard, J. H.; Heijnen, H. J.; Rops, R. H.; Cools, A. R.; Van Rossum, J. M. Separation of inhibiting and stimulating effects of morphine on self-stimulation behavior by intracerebral microinjections. Eur. J. Pharmacol. 36:443–446; 1976.
4. Heikkila, R. E.; Manzino, L. Behavioral properties of GBR-12909, GBR-13069 and GBR-13098: Specific inhibitors of dopamine uptake. Eur. J. Pharmacol. 103:241–248; 1984.
5. Hoffman, I. Pharmacology of nomifensine. Int. Pharmacopsychiatry 17(Suppl. 1):4–20; 1982.
6. Holmes, L. J.; Wise, R. A. Contralateral circling induced by tegmental morphine: Anatomical localization, pharmacological specificity and phenomenology. Brain Res. 326:19–26; 1985.
7. Iyengar, S.; Kim, H. S.; Wood, P. L. Agonist action of the agonist/antagonist analgesic butorphanol on dopamine metabolism in the nucleus accumbens of the rat. Neurosci. Lett. 77:226–230; 1987.
8. Iwamoto, E. T.; Way, E. L. Circling behavior and stereotypy induced by intranigral opiate microinjections. J. Pharmacol. Exp. Ther. 203:347–359; 1977.
9. Jenck, F.; Gratton, A.; Wise, R. A. Opposite effects of ventral tegmental and periaqueductal gray morphine injections on lateral hypothalamic stimulation-induced feeding. Brain Res. 399:24–32; 1986.
10. Jenck, F.; Gratton, A.; Wise, R. A. Opioid receptor subtypes associated with ventral tegmental facilitation of lateral hypothalamic brain stimulation reward. Brain Res. 423:34–38; 1987.
11. Joyce, E. M.; Iversen, S. D. The effect of morphine applied locally to the mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci. Lett. 14:207–212; 1979.
12. Kaakkola, S. Contralateral circling behavior induced by intranigral injection of morphine and enkephalin analogue FK 33-824 in rats. Acta Pharmacol. Toxicol. 47:385–393; 1980.
13. Matthews, R. T.; German, D. C. Electrophysiological evidence for excitation of rat ventral tegmental dopaminergic neurons by morphine. Neuroscience 11:617–626; 1984.
14. Ostrowski, N. L.; Hatfield, C. B.; Caggiula, A. R. The effects of low doses of morphine on the activity of dopamine containing cells and on behavior. Life Sci. 31:2347–2350; 1982.
15. Phillips, A. G.; LePiane, F. G. Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacol. Biochem. Behav. 12:965–968; 1980.
16. Rompre, P.-P.; Wise, R. A. A study of the interactions of pimozide, morphine and muscimol on brain stimulation reward: Behavioral evidence for depolarization inactivation of A10 dopaminergic neurons. Ann. NY Acad. Sci.; in press.
17. Wise, R. A.; Holmes, L. Circling from unilateral morphine: Direction is controlled by environmental stimuli. Brain Res. Bull. 16:267–269; 1986.