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Reexpression of Morphine-Induced Oral Stereotypy Six Months After Last Morphine Sensitizing Dose

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POLLOCK, J. AND C. KORNETSKY. *Reexpression of morphine-induced oral stereotypy 6 months after last morphine sensitizing dose.* PHARMACOL BIOCHEM BEHAV **53**(1) 67–71, 1996.—We describe three experiments, two of which were designed to determine the duration of the sensitization to morphine-induced oral stereotypy caused by three high doses of morphine (MS) administered 12 h apart (10, 20, and 20 mg/kg). In one of these experiments, we also administered an additional 40 mg/kg. The third experiment was designed to determine the role of mode of drug administration in the development of MS-induced sensitization; drug was delivered by an implanted minipump. By the third high dose, all rats manifested marked repetitive gnawing and biting behavior. The stereotypy was reexpressed up to over 300 days in repeatedly challenged animals and up to at least 180 days in animals receiving only one MS challenge. The development of sensitization was not altered by the mode of drug administration.

Opioids Gnawing behavior

CHRONIC administration of high doses of morphine sulfate (MS) in rats often elicits a variety of stereotypic behaviors. Among these behaviors is compulsive gnawing, in which the animal bites an object in the experimental chamber (e.g. floor bars) or itself (e.g., digits) (11,15). The biting behavior may be so intense that self-wounding or even amputation can occur (6). Animals will initially combine grooming, sniffing, and biting behaviors; however, with repeated doses of an opiate they will bite exclusively (6). In addition to the rat, opiate-induced oral stereotypies have been found in guinea pigs (2), hamsters (17), and primates (3). Opiate-induced stereotypy is not confined to MS. Robust biting behavior has been reported following the administration of methadone (2,3), heroin (5), and the μ-receptor agonist morphiceptin (12).

The gnawing behavior associated with MS administration has been attributed to the excessive release of catecholamines in mesolimbic (9), nigrostriatal (8), and ventral thalamic regions of the rat brain (1). This MS-induced stereotypy can be blocked, in a dose-dependent manner, by the D_1-dopamine antagonist SCH-23390, but not by the D_2-antagonist raclopride (15). Further evidence suggesting a dopamine-opioid interaction is the finding that naloxone blocks the stereotypy caused by low-dose dopamine agonist in rats previously treated with dopamine antagonists (16). Of major importance is that the oral stereotypy of the rat, once established by the administration of four high doses of MS for 36 h, 10, 20, 20, and 40 mg/kg, subcutaneously (SC), was reexpressed by the administration of 4.0 mg/kg of MS 30 days after the initial MS exposure (15).

This report describes three experiments designed to characterize the duration of the developed sensitization to the stereotypic effects of MS, and the possible role of the mode of administration on the development of the sensitization.

EXPERIMENT 1

The purpose of this experiment was to determine the duration of the sensitization to the stereotypic effects in animals initially treated with four high doses of MS administered at 12-h intervals within 36 h.

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Methods

We used five F-344 male rats (Charles River Laboratories, Wilmington, MA) weighing 325–350 g. Rats were housed individually, received water and standard rat chow *ad libitum*, and were kept on a 12 L : 12 D cycle. All experiments were carried out during the light portion of the cycle.

MS was dissolved in isotonic saline, prepared in a volume of 1 mg/ml, and administered SC. All rats were administered four doses of MS (10, 20, 20, and 40 mg/kg) equally spaced over a 36-h period. Following each MS injection, animals were placed in an acrylic observation chamber (21 × 21 × 35 cm high) with a grid floor composed of horizontal steel bars 2.0 mm in diameter and separated by 10 mm. Rats were observed for a 3-h period for incidence of oral stereotypy. The criterion of a positive occurrence of oral stereotypy was preselected as a period of at least 5 consecutive min of chewing behavior directed at either objects in the test chamber, or self-directed.

A low-dose challenge of MS (4.0 mg/kg) was administered to all animals at various time periods after the initial administration of the three high doses. From days 30–56, animals were administered five to nine spaced MS challenges. On days 90 and 180, animals were once again challenged. Four or five spaced challenges were given from days 270–303. Finally, three of the five animals received two to three MS challenges from days 360–448.

Results

The first high dose of MS (10 mg/kg) resulted in sedation in all subjects lasting from 90–150 min. Although no stereotypy was observed in any animals during the 180-min observation period, one rat was incidentally observed to exhibit marked stereotypy at approximately the 190th min after the administration of the first dose of morphine. Four of the five rats exhibited significant chewing behavior after the second dose. All rats (n = 5) exhibited significant chewing behavior after the third and fourth dose. Table 1 shows the latency of onset of stereotypy for each animal for each of the doses except for the fourth one (40 mg/kg). Although stereotypy was observed after the 40-mg/kg dose, latency was not systematically recorded. Table 1 indicates a progressive decrease in latency to onset of the stereotypy.

Table 2 shows the incidence of stereotypy to the 4.0-mg/kg challenge doses of MS. As indicated, each challenge with MS resulted in significant gnawing behavior, with a minimum of 5 min of continuous gnawing or biting, at each time period tested in every rat. In three of the rats (nos. 1, 3, and 4), 4 mg/kg of MS resulted in significant gnawing behavior 360–448 days after the four high sensitizing doses. Initially, all rats directed their chewing behavior toward the floor bars of the observation chamber. After repeated challenges, the gnawing-chewing behavior became self-directed. Most commonly, the rats chewed on their forelegs, front foot pads, and nails. In some cases, during the later challenges with MS, the chewing resulted in hair removal and/or self-mutilation. If the latter occurred, the animal was killed with an overdose of pentobarbital. Also shown in Table 2 is the mean latency for all MS challenges for each animal to the onset of stereotypy after the 4.0-mg/kg challenge doses. A comparison of this latency to that illustrated in Table 1 shows that the latency was shorter after the challenge doses than after higher sensitizing doses.

TABLE 1

LATENCY TO ONSET (min) OF MORPHINE-INDUCED ORAL STEREOTYPY FOR EACH OF FIVE RATS AFTER THE INITIAL FIRST THREE HIGH-MORPHINE DOSES

Animal	10 mg/kg	20 mg/kg	20 mg/kg
1	–	–	37
2	–	171	60
3	–	68	30
4	–	83	54
5	–	95	45

–, Animal did not exhibit stereotypy during the duration of the observation time.

EXPERIMENT 2

Because the group of animals used in Experiment 1 received repeated MS challenge doses that resulted in repetitive biting behavior, it cannot be determined whether the persistent effect was due to the initial high doses or continuous priming of the animal with the 4.0-mg/kg dose. In Experiment 2, rats were challenged with MS only once after the initial high-dose MS treatments. Rats in a control group that received saline in lieu of the high doses of MS also received the later challenge of a low dose of MS.

Methods

We used 24 male F-344 rats, weighing 325–350 g, in this experiment. Housing conditions and preparation were the same as in Experiment 1. Animals were randomly assigned to a three-high-dose MS treatment group (n = 12) or a saline treatment group (n = 12) and observed for stereotypy. The administration of each of the three doses of MS (10, 20, and 20 mg/kg) or saline were spaced 12 h apart. Then, 30 days later, four rats from the MS group and four rats from the saline group were administered 4.0 mg/kg MS and observed, as in Experiment 1, for presence of oral stereotypy. This procedure was repeated with another group of eight animals at 90 days, and in a final group of eight rats at 180 days following the last injection of MS or saline. Ratings of the presence of stereotypy were carried out with the observer blinded as to the initial treatment.

Two-tailed Fisher's exact test was performed comparing the presence or absence of oral stereotypy in the MS vs. saline (initial treatment) groups at 30, 60, and 180 days.

Results

All 12 animals that received the three high doses of MS exhibited marked oral stereotypy by the third high dose, with five of the 12 animals reaching the criterion of 5 consecutive min of oral stereotypy during the second MS exposure. Table 3 presents the incidence of oral stereotypy in each group resulting from the 4.0-mg/kg MS challenge. As indicated, all animals initially treated with the three high doses of MS exhibited oral stereotypy when challenged with 4.0 mg/kg MS. The onset of chewing and gnawing behavior was 14–46 min after the administration of 4.0 mg/kg MS.

The dose of 4.0 mg/kg MS in the control rats initially treated with saline caused a substantial decrease in activity that was eventually followed by an increase in activity, characterized by grooming, sniffing, and locomotion. Although no control rat met the criterion of 5 consecutive min of biting behavior, two of the four rats showed evidence of this behavior. In one animal the behavior lasted 1–2 min, and in the

TABLE 2

NUMBER OF TIMES EACH ANIMAL REEXPRESSED THE ORAL STEREOTYPY AFTER A MORPHINE CHALLENGE (4.0 mg/kg) AT VARIOUS TIME PERIODS FOLLOWING FOUR SENSITIZATION DOSES AS WELL AS THE MEAN ± SEM LATENCY TO ONSET OF STEREOTYPY FOR EACH ANIMAL

	Day					
Animal	30–56	90	180	270–303	360–448	Mean Latency (min)
1	9(9)*	1(1)	1(1)	5(5)	2(2)	26.9 ± 4.5
2	9(9)	1(1)	1(1)	5(5)		23.3 ± 2.1
3	9(9)	1(1)	1(1)	5(5)	2(2)	27.4 ± 3.8
4	9(9)	1(1)	1(1)	4(4)	3(3)	21.4 ± 7.0
5	9(9)	1(1)	1(1)	4(4)		35.5 ± 4.0

*Numbers in parentheses indicate the number of challenges at the indicated day(s) following the sensitizing MS doses.

other 3–4 min, and thus did not meet the criterion of 5 consecutive min. Because no drug-naive rat had previously shown oral stereotypy in response to the low dose of MS, a second MS injection was given 3 days later to both the experimental and control rats. One of the two control rats that had exhibited the biting behavior again began to chew on its forepaws for a full minute, breaking for approximately 13 min and then returning to self-directed chewing behavior. The oral stereotypy, however, did not meet the 5-min criterion. In this repeated challenge in the same four rats, none of the other control animals exhibited biting behavior after 4.0 mg/kg MS, whereas all of the experimental animals reexpressed the stereotypy.

EXPERIMENT 3

It has been suggested (13) that the enhancement of the stereotypic behaviors following daily injections of amphetamine is the result of the brief period of increased activity, followed by the daily rebound decreases in activity. Also, it is possible that the injection of MS during the high-dose administration became a conditioned stimulus that led to the stereotypy when later low doses of MS were injected. To determine whether the mode of morphine administration of the high doses of MS was a significant variable accounting for the reexpression of the stereotypy after the administration of a low dose of MS, we performed the following experiment. In this test, the high-dose regimen was given via an osmotic minipump.

TABLE 3

INCIDENCE OF MORPHINE-INDUCED ORAL STEREOTYPY IN RATS THAT RECEIVED NO MORPHINE TREATMENT BETWEEN INITIAL SENSITIZATION AND A 4.0-mg/kg CHALLENGE DOSE AT INDICATED POSTSENSITIZATION DAY

	Day Postsensitization		
Initial Treatment	30	90	180
MS (n = 12)	4(n = 4)*	4(n = 4)*	4(n = 4)*
Sal (n = 12)	0(n = 4)	0(n = 4)	0(n = 4)†

*p = 0.03 (Fisher exact probability test).

†Although stereotypy was observed in two of the rats, the criterion of 5 consecutive min was not met.

Methods

Four naive F-344 male rats weighing 325–350 g were anesthetized with halothane and implanted with an MS-containing osmotic minipump (Alza, Palo Alta, CA). The pumping rate was 8.63 ± 0.18 μl/h, and the pumps were filled with 2 ml MS (53 mg/ml). Then, 36 h later, the rats were reanesthetized and the pumps removed. Following each surgery, wound clips were used to secure the incision and Panalog (E.R. Squibb & Sons, Princeton, NJ) ointment was applied.

Immediately following the implantation of the minipump, the rats were placed in the observation chamber with the steel bar floors and were observed for 3 h for the incidence of stereotypy. Following the observation period, the animals were returned to their home cages. Then, 20 h following implantation, the animals were again observed for stereotypy. We removed the pumps 36 h after minipump implantation, and observed the rats again for the presence of stereotypy. After 24 h and 30 days, the animals were challenged with a 4.0-mg/kg, SC, dose of MS and observed for the presence of stereotypy.

Results

For the first 3 h after the implantation of the minipump, the rats appeared to be sluggish and unresponsive to even loud auditory stimuli, and showed no evidence of oral stereotypy. Because in our experience, 30 min after halothane anesthesia the behavior of the F-344 rats appears to be normal, only the first 30 min can be attributed to the halothane. At 20 h postimplantation, all four rats showed increase in locomotor activity, characterized by rearing and walking about the cage; however, there were no signs of oral stereotypic behavior. At 36 h, there was still no evidence of stereotypy and the minipumps were removed.

A total of 24 h after minipump implantation, the animals were challenged with a 4.0-mg/kg injection of MS. Within the first 5 min, there was a brief increase in locomotor behavior in all animals, followed by approximately 60 min of sedation. At 65–75 min after the MS injection, three of the four animals began to chew on their forepaws. The fourth animal chewed on its foot pads and toenails at 120 min postinjection. All chewing behavior exceeded the 5-min criterion.

At the 30-day postminipump challenge with 4.0 mg/kg MS, three of the animals showed significant self-directed oral stereotypy within 20 min of the injection. The fourth rat, the one that showed the latent MS-induced chewing behavior 24 h

after pump removal, did not show signs of stereotypy throughout the 3-h observation period.

DISCUSSION

These experiments replicate and extend a previous report (15) that the administration of high doses of MS—in this case as few as four—administered within a 24-h period to the F-344 rat results in the development of marked oral stereotypy characterized by compulsive biting and gnawing behavior, which can be reexpressed 30 days later by the administration of low-dose MS.

The results obtained in Experiment 1 suggest that the sensitization caused by four high doses of MS may last for up to 14 mo after the last high dose. However, the rats used in that experiment received numerous injections (up to 18) of 4.0 mg/kg MS during the 14-mo period. Thus, it was possible that the sensitization was maintained for that length of time by repeated MS injections. Experiment 2, however, indicates that the sensitization can last up to 6 mo in animals that had no interpolated MS treatment between the high-dose experience and the 4.0-mg/kg challenge dose.

Evidence that MS-induced oral stereotypy sensitization is independent of nonpharmacologic interactions is suggested in the results of Experiment 3, in which the initial high doses of MS were administered by constant infusion via an osmotic minipump. The animals in the minipump experiment did not exhibit stereotypic behavior during the 36 h when the pump was in place. All four of the rats, however, manifested significant stereotypy when challenged with 4.0 mg/kg of MS 24 h after the removal of the pump, and three rats showed the stereotypy when challenged with 4.0 mg/kg of MS 30 days after the pump removal. These results indicate that it is not necessary for the animals to experience the initial stereotypy resulting from the repeated high-dose injections for sensitivity to develop to this MS effect.

Although the failure of one animal suggests that the sensitization is less robust when induced by the maintenance of a steady infusion of MS, it is also possible that the animal failing to respond may not have received adequate initial dosing. The osmotic minipump delivered approximately 16.5 mg of MS during the implantation period. Although this amount was slightly less than the approximate 17.5 mg of MS delivered by the intermittent injection procedure, many animals showed marked stereotypy after only two of the initial injections of MS (approximately 10.5 mg). If the pump was not adequately primed, or was placed in a bed of connective tissue rather than against a better absorption surface, the amount of MS that diffused from the pump may have been suboptimal.

The mechanisms involved in the sensitization of these effects have yet to be elucidated. The expression of these movements in the rat can be blocked by the D_1 antagonist SCH-23390 (15), although it cannot block the development of sensitization (10). The NMDA antagonist, MK-801, however, blocks not only the expression of the stereotypy but also the development of sensitization (10), suggesting a role for glutamate in opioid sensitivity. The extent of overlap between the psychomotor excitation and the stereotypy caused by opioids is not completely known. However, our data indicate that the stereotypy is not conditioned by the environment, and thus constitutes a direct pharmacologic action that is independent of the psychomotor effects of the opioids.

In addition to the fact that sensitization occurs, some of the results obtained in these experiments suggest a process that is similar to a kindling effect. In a review of the phenomenon of kindling by Goddard (7), it has been defined as a ". . . change in brain function caused by repeated focal stimulation that results in a lasting predisposition to epileptiform convulsions." Our results, although not related to epileptiform seizures, seem to fit the definition of kindling as described by Goddard. That is, the stereotypy is not manifested after the first high dose and may not be seen in some animals after the second; however, it appears more readily and is more intense by the third high dose. Although this could be a cumulative effect of MS, the even shorter latency to the challenged doses (Table 2) suggests that it is not a simple function of cumulation of drug in the animal. Also, in Experiment 3, the animals did not manifest stereotypic behavior during the 36 h of exposure to MS via the minipump, although they showed sensitization when later challenged with 4.0 mg/kg MS. In a previous experiment (15), sensitization could be induced not only by the high doses of MS but also by coadministration of electrical stimulation to the medial forebrain bundle and an injection of 4.0 mg/kg MS. This biting behavior was only manifested after a number of spaced exposures to brain stimulation in the presence of 4.0 mg/kg MS.

It is interesting that there are spontaneous oral stereotypies that occur naturally in animals under some conditions. These stereotypies resemble those seen after the high doses of MS but can be blocked by opioid antagonists. Horses housed in stalls often display stereotypic biting directed against themselves or the boards of the stall (4). Dogs and pigs will also exhibit aggressive self-biting behavior. The blocking of these abnormal repetitive movements by opioid antagonists suggests that the syndrome may result from the stress-induced release of endogenous opioids (4,14).

In summary, the results of these experiments indicate that repeated high-dose MS treatment in the rat causes a long-lasting sensitization of stereotyped biting and gnawing behavior. This sensitization is independent of the mode of administration of the initial high doses of MS.

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