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Tolerance and Sensitization to the Biphasic Effects of Low Doses of Morphine in the Hamster

PAUL SCHNUR, FRANCINE BRAVO AND MARK TRUJILLO

Center for Psychology and Mental Health, University of Southern Colorado, Pueblo, CO 81001

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SCHNUR, P., F. BRAVO AND M. TRUJILLO. *Tolerance and sensitization to the biphasic effects of low doses of morphine in the hamster.* PHARMACOL BIOCHEM BEHAV 19(3) 435–439, 1983.—Two experiments investigated the dose and time related effects of morphine sulfate on wheel running behavior in golden Syrian hamsters. In Experiment 1, within-subject comparisons were made of the acute effects of 5, 20, and 40 mg/kg doses of morphine sulfate on running wheel activity. Compared with saline, morphine produced a dose related decrease in activity followed by a dose related recovery. At the lowest dose, the time effect curve was biphasic, with sustained hyperactivity following the recovery. In Experiment 2, the effects of low doses of morphine sulfate (0.5, 1.0, 2.5, 5.0 mg/kg) on running wheel activity were monitored for three days. Biphasic time effect patterns were evident at each dose: An initial period of hypoactivity was followed by recovery and subsequent hyperactivity. Moreover, repeated administration produced both tolerance and sensitization to morphine's effects on activity. Implications for mechanisms underlying the biphasic response pattern are discussed.

Hamsters Morphine Tolerance Sensitization Locomotor activity Opiates

RECENTLY, we reported that morphine has biphasic effects on running wheel activity in hamsters [10]. Compared with saline controls, a 5 mg/kg dose of morphine sulfate caused sustained hyperactivity; higher doses (10, 20, and 40 mg/kg) produced an immediate dose-related hypoactivity followed by a gradual dose-related recovery and finally a period of hyperactivity. Such findings are of interest because they indicate that the hamster's response to morphine is not exceptional [16]. Rather, the effects of morphine on locomotor activity in the hamster are very similar to morphine effects reported in the rat [1]. In addition, the demonstration of sequential depressant and stimulant actions of morphine in the hamster is consistent with the thesis advanced by Domino and his co-workers [5] that morphine has both excitatory and inhibitory effects in all species. Indeed, when care is taken to ensure an adequate behavioral baseline, biphasic effects can be demonstrated even in mice [8], an animal generally thought to show only hyperactivity to morphine.

The present work continues our investigation of morphine actions on locomotor activity in the hamster. The first experiment was designed to demonstrate that biphasic morphine effects could be found under different conditions than those described in our previous report. The second experiment investigated the effects of low doses of morphine sulfate on locomotor activity and monitored changes in those effects for three days.

EXPERIMENT 1

The purpose of the first experiment was to test the effects of four doses of morphine sulfate (0, 5, 20, 40 mg/kg) on running wheel activity in hamsters. In our previous work with these doses [10], experimentally naive hamsters were used in between-groups designs that assigned animals matched for baseline activity rates to the various drug treatments. In the present experiment, animals with prior drug and running wheel experience were tested in a within-groups design. Furthermore, the previous study measured the hamster's daily response to morphine for eight days and biphasic time-effect patterns were based on the accumulated data. In the present experiment, the effects of each morphine sulfate dose were assessed in a single two hour session.

METHOD

Subjects

Twenty-four adult golden Syrian hamsters (11 males, 13 females), with a mean weight of 129.5 grams were used. The hamsters were descended from animals obtained from Sasco, Inc. (Omaha, NE). They were housed individually in stainless steel cages, maintained on a 12:12 hour light-dark cycle, and given free access to food and water throughout the experiment. All animals had served in pilot studies two months prior to the present experiment and thus had previous exposure to the running wheel and to the effects of morphine.

Apparatus and Materials

The apparatus consisted of eight identical activity wheels (Wahmann Co., Model LC-34) which were housed in a room dimly illuminated by two 15 watt bulbs. Running wheels were fitted with microswitches and interfaced to Canon

printing calculators (Model TP-8), modified [4] to record the number of wheel revolutions. An ambient noise level of 79 dB (re: 0.0002 dynes/cm², A scale) was maintained.

Morphine injections consisted of 5, 20 or 40 mg/kg doses of morphine sulfate, expressed as the salt, dissolved in 1 ml of physiological saline. Morphine and saline injections were administered in 1 ml/kg volumes.

Procedure

Four test sessions, spaced approximately one week apart, were conducted. At weekly intervals, animals were removed from their home cages, weighed and transported to an adjoining room where they received a subcutaneous injection in the dorsal surface of the neck of either saline or the appropriate dose of morphine. The animals were then left undisturbed in their cages for fifteen minutes before being placed in the running wheels for a two hour session. The number of wheel revolutions was recorded every twenty minutes for each animal. All animals received drugs in the same randomly chosen order: 40 mg/kg (Group MS-40), 5 mg/kg (Group MS-5), 0 mg/kg (Group Saline), 20 mg/kg (Group MS-20).

RESULTS AND DISCUSSION

Figure 1 shows mean activity (number of wheel revolutions) for the two hours following drug administration for all conditions. Compared with saline controls, animals receiving morphine showed an initial dose-related depression in locomotor activity, followed by a dose-related recovery. This recovery was especially rapid in group MS-5 so that for most of the two hour session Group MS-5 showed sustained hyperactivity. Group MS-20 also showed complete locomotor recovery by the end of the two hour session, though evidence of hyperactivity appears to have been precluded by the termination of the session. Locomotor activity in Group MS-40 was depressed for the entire two hour session, with partial recovery evident towards the end of that period. These impressions are corroborated by 4×6 repeated measures analysis of variance (ANOVA) which indicated that the effects of dose, $F(3,69)=84.55$, $p<0.001$, minutes, $F(5,115)=32.18$, $p<0.001$, and the interaction between dose and minutes, $F(15,345)=11.36$, $p<0.001$, were all significant. Furthermore, an analysis of simple effects indicated that, compared with Group Sal, Group MS-5 was hypoactive after 20 minutes, $F(1,345)=11.62$, $p<0.001$, but hyperactive after 60 minutes, $F(1,345)=6.54$, $p<0.025$, after 80 minutes, $F(1,345)=3.90$, $p<0.05$, after 100 minutes, $F(1,345)=8.01$, $p<0.005$, and after 120 minutes, $F(1,345)=4.82$, $p<0.05$.

In agreement with our earlier data [10], the present results indicate that morphine produces reliable effects on locomotor activity in hamsters. In the two hours following drug administration, morphine's time-effect curve varied as a function of dose. A 5 mg/kg dose of morphine produced a biphasic time effect pattern, whereas higher doses produced dose related decreases in locomotor activity followed by dose-related rates of recovery. The fact that such effects were evident in a single two hour session among animals with various pre-experimental histories attests to their robust nature and should dispel any doubts about the hamster's sensitivity to morphine. In fact, sedative effects observed in this experiment were somewhat greater than those reported earlier [10]. That is, in our previous work, where time-effect data were accumulated for eight days, we did not observe an initial decrease in activity at a dose of 5 mg/kg; that dose appeared to produce hyperactivity only. Similarly.

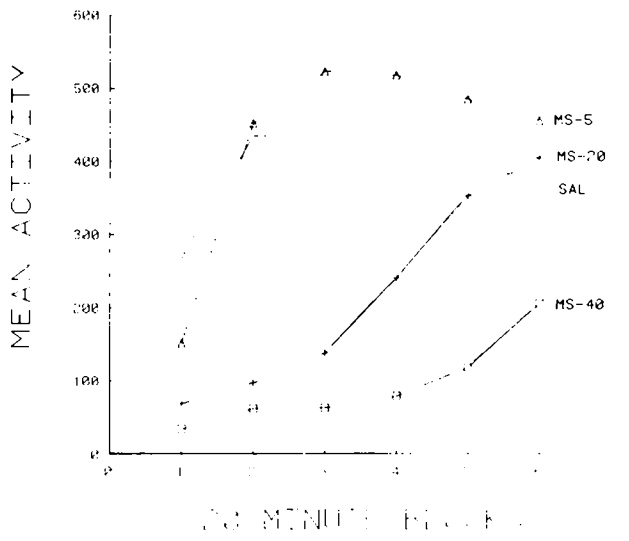

FIG. 1. Mean activity (number of wheel revolutions) as a function of 20-minute time blocks for all groups in Experiment 1.

in our previous work, recovery from the suppressive effects of a 20 mg/kg dose of morphine was complete in little more than an hour and hyperactivity was apparent within two hours; a more rapid recovery and shift to hyperactivity than observed here. It is possible that some tolerance had developed to the sedative effects of morphine during the eight days of our earlier study. Perhaps experience with morphine increases the rate of recovery from hypoactivity and decreases the time-to-onset of hyperactivity.

EXPERIMENT 2

The purpose of Experiment 2 was to investigate the effects of low doses of morphine on locomotor activity in the hamster and to monitor the changes that occur with repeated morphine administration. In this experiment, we were interested in replicating the biphasic time-effect pattern observed in the first experiment with a 5 mg/kg dose of morphine. Furthermore, we wanted to test the hypothesis that the initial hypoactivity would give way to hyperactivity as a function of experience with the drug. Although tolerance to the sedative effects of morphine is well documented, tolerance per se implies only that hypoactivity would decrease, not that it would be replaced by hyperactivity. Finally, we wanted to explore the effects of low doses of morphine in the expectation of finding one or more doses that are purely excitatory in their effects. Low doses of morphine (5 mg/kg or less) have been reported to produce hyperactivity in rats [1,5]. In Experiment 2, the effects of daily injections of morphine sulfate (0.5, 1.0, 2.5 and 5.0 mg/kg) were monitored for two hours on each of three successive days.

METHOD

Subjects

Thirty adult golden Syrian hamsters (14 males, 16 females), with a mean weight of 101.7 grams were used. Eleven hamsters were obtained from Sasco, Inc. (Omaha, NE); the other 19 were descended from Sasco hamsters.

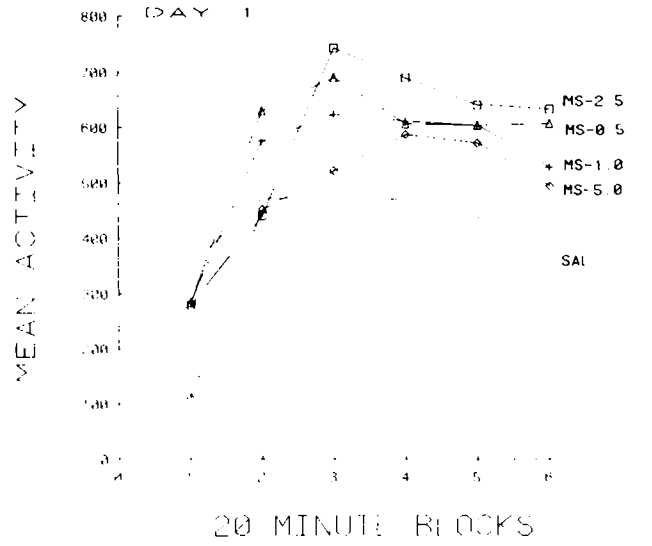

FIG. 2. Mean activity (number of wheel revolutions) as a function of 20-minute time blocks for all groups on Day 1 in Experiment 2.

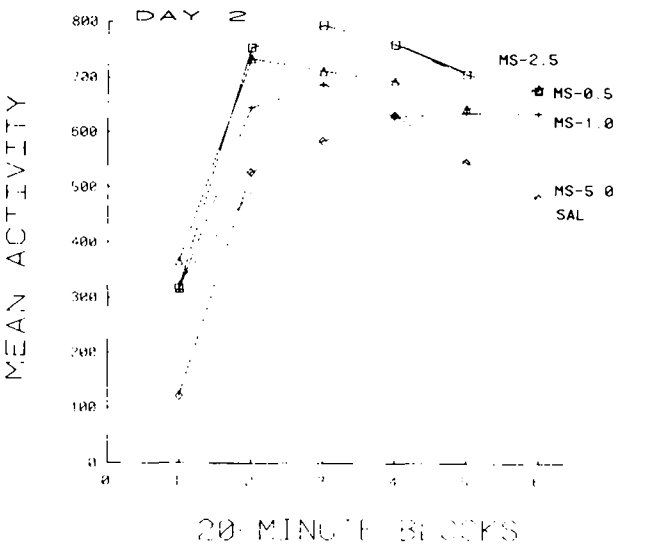

FIG. 3. Mean activity (number of wheel revolutions) as a function of 20-minute time blocks for all groups on Day 2 in Experiment 2.

Animals were housed individually in stainless steel cages, maintained on a 12:12 hour light-dark cycle, and given free access to food and water throughout the experiment.

Apparatus and Materials

The apparatus was the same as that used in Experiment 1 except that the ambient noise level was maintained at 74 dB (re: 0.0002 dynes/cm^2, A scale) by a Lafayette white noise masking package (Model 15800). Morphine injections consisted of 0.5, 1.0, 2.5, or 5.0 mg/kg doses of morphine sulfate, expressed as the salt, dissolved in 1 ml of saline. Morphine and saline injections were administered in 1 ml/kg volumes.

Procedure

Experimental procedures took place on six consecutive days. On the first three days, animals were weighed, injected with saline and placed in the running wheel for a two hour baseline session. The number of wheel revolutions was recorded at 20 minute intervals for each animal. Baseline sessions served to accustom the animals to the running wheel and to the injection procedure. In addition, baseline data were used to create five groups of hamsters matched on mean activity level. These groups were then randomly assigned to five drug treatment conditions (n=6) for three test days: Group Sal (saline controls), Group MS-5.0 (5 mg/kg morphine sulfate), Group MS-2.5 (2.5 mg/kg morphine sulfate), Group MS-1.0 (1 mg/kg morphine sulfate) and Group MS-0.5 (0.5 mg/kg morphine sulfate). On test days, animals were weighed, injected with saline or the appropriate dose of morphine and, following a 15 minute respite, placed in the running wheels for a two hour session. The number of wheel revolutions was recorded at 20 minute intervals for each animal.

RESULTS AND DISCUSSION

Figure 2 shows mean activity as a function of time for all groups on the first test day. The effects of morphine on activity were a function of both dose and time since injection. Compared with saline, all doses of morphine produced an immediate decrease in activity, followed by recovery, and subsequent hyperactivity. Thus, the biphasic pattern we reported for high doses of morphine [10] and for a 5 mg/kg dose in Experiment 1 has now been demonstrated for low doses, as well. Nevertheless, there are some differences between the effects of high and low doses: At high doses, the immediate decrease in activity varied directly with dose and the rate of recovery varied inversely with dose. At the low doses tested here, the dose-effect relations were more complex: The largest decrease in activity was produced by a 5 mg/kg dose, but differences in hypoactivity among other doses were negligible. Similarly, the rate of recovery from hypoactivity was not simply ordered from lowest to highest dose. Although Groups MS-0.5 and MS-1.0 recovered before Groups MS-2.5 and MS-5.0, Group MS-2.5 became more hyperactive than all other morphine groups.

Figures 3 and 4 show mean activity as a function of time for all groups on the second and third test days, respectively. On Test Days 2 and 3, morphine continued to exert powerful effects on activity, while several interesting changes emerged as a function of days. For example, whereas all groups showed a biphasic time-effect curve on Day 1 the initial hypoactivity decreased for all groups across days, so that on Days 2 and 3 only Group MS-5 continued to show a biphasic response pattern. And whereas Group MS-2.5 was hypoactive in the first 20 minutes of the Day 1 test session, it was hyperactive in the first 20 minutes of the Day 3 session. Clearly, experience with low doses of morphine produced tolerance to morphine's sedative effects.

However, experience with morphine also had a sensitizing effect. As noted above, for example, the initial response of Group MS-2.5 on Day 1 was hypoactivity; on Day 3, the initial response of Group MS-2.5 was hyperactivity. That is, Group MS-2.5 was not merely tolerant to morphine—it responded to the third morphine injection with an immediate increase in activity. A similar though smaller change can be detected in the initial responding of Group MS-0.5 on Test

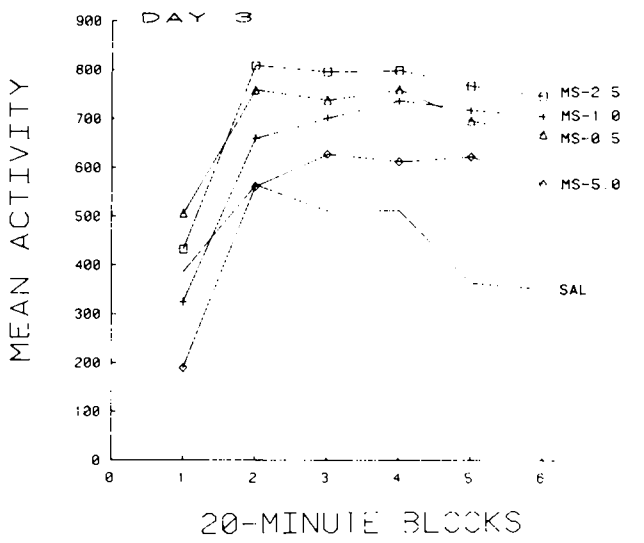

FIG. 4. Mean activity (number of wheel revolutions) as a function of 20-minute time blocks for all groups on Day 3 in Experiment 2.

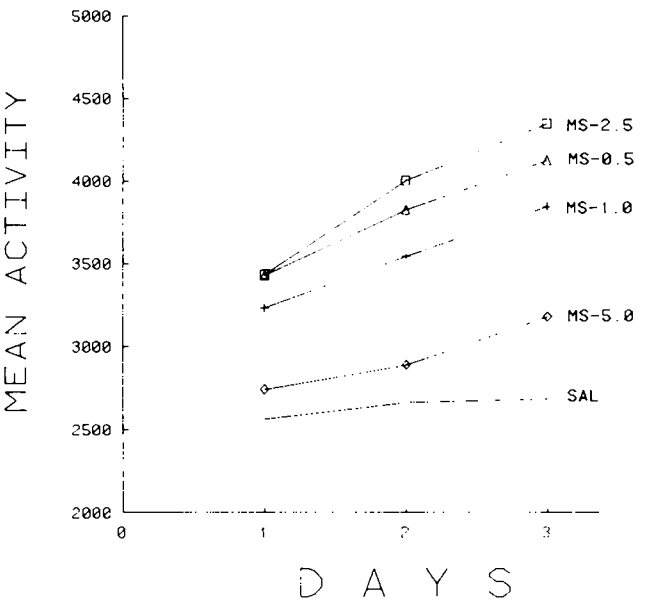

FIG. 5. Mean activity (number of wheel revolutions) as a function of days for all groups in Experiment 2.

Days 1, 2 and 3. Sensitization to morphine's excitatory effects is evident also in the amount of hyperactivity and in its decreasing latency on successive test days. Again, Group MS-2.5 is exemplary. On Day 1, Group MS-2.5 attained a peak of activity of 744 revolutions in the third time period of the test session; on Day 2, it attained a higher peak of activity (793 revolutions) in the same period of time; and on Day 3, it attained a still higher peak of activity (808 revolutions) 20 minutes earlier. Similar changes can be seen in the other morphine groups and in Fig. 5 which shows mean activity across days for all groups. The increase in mean activity in the morphine groups across days reflects both tolerance to morphine's sedative effects and sensitization to morphine's excitatory effects. Of course, our use of the terms tolerance and sensitization is purely descriptive and is not intended to imply anything specific about underlying mechanisms.

These conclusions are substantiated by a 5 (Dose) × 3 (Days) × 6 (Minutes) mixed factorial ANOVA. The main effects of dose, $F(4,25)=3.11$, $p<0.05$, of days, $F(2,50)=21.30$, $p<0.001$, and of minutes, $F(5,125)=65.69$, $p<0.001$, were all significant. The interactions between dose and minutes, $F(20,125)=2.60$, $p<0.001$, between days and minutes, $F(10,250)=1.99$, $p<0.05$, and between dose, days and minutes, $F(40,250)=1.48$, $p<0.05$, were all significant. In addition, during the first time period of Day 1, Group Sal was more active than the average of Groups 0.5, 1.0 and 2.5, $F(1,250)=3.08$, $0.05<p<0.10$, and more active than Group 5.0, $F(1,250)=24.35$, $p<0.001$. Group 5.0 was hypoactive compared with Groups 0.5, 1.0 and 2.5, $F(1,250)=18.40$, $p<0.001$. Finally, during the first time period of Day 3, Group 0.5 was hyperactive compared with Group Sal, $F(1,250)=5.69$, $p<0.025$, whereas Group 5.0 was still hypoactive, $F(1,250)=16.18$, $p<0.001$.

GENERAL DISCUSSION

The present study confirms and extends our earlier observations concerning the effects of morphine on locomotor activity in hamsters [10]. We reported that for 10, 20 and 40 mg/kg doses, morphine produces a biphasic time-effect pattern on motor activity in the running wheel. The present work indicates that this biphasic pattern is elicited also by low doses of morphine: 5 mg/kg (Experiments 1 and 2), 2.5 mg/kg, 1 mg/kg and 0.5 mg/kg (Experiment 2). Moreover, the present study indicates that experience with low doses of morphine affects both portions of the biphasic response pattern. In Experiment 2, repeated administration of morphine produced tolerance to the initial hypoactive phase and sensitization to the subsequent hyperactive phase. Whether similar changes occur as a result of experience with higher doses of morphine is currently under investigation in our laboratory. In rats, Babbini *et al.* [2] reported that following chronic treatment with a 20 mg/kg dose of morphine, sedative effects disappeared, excitatory effects increased, and the latency to peak activity decreased.

The fact that hamsters respond to low doses provides confirming evidence that hamsters are not insensitive to the sedative effects of morphine [7,16]. In a recent paper, Ostrowski *et al.* [9] reported dose-dependent effects of morphine in the hamster, but concluded that large doses of morphine (40 mg/kg or more) are necessary for producing reliable motor impairments. The present work establishes that running wheel activity can be impaired at much lower doses. Indeed, in Experiment 2, sedation was observed at all doses; none had purely excitatory effects. By contrast, the effects of low doses of morphine in the rat are reported to be excitatory [1,5]. However, most such reports do not provide detailed time-effect analyses. Typically, [1,5] morphine's effects are measured once per hour. Had we measured the hamster's responding only once per hour, the initial sedation would have been obscured by the subsequent hyperactivity and low doses would have appeared to be purely excitatory. Perhaps, morphine at effective doses in all species produces an initial, transient phase of hypoactivity (cf., [5]).

A number of hypotheses have been proposed to account

for the mixed depressant and stimulant actions of morphine. For example, biphasic effects have been attributed to different drug-receptor interactions, one producing sedation, the other excitation [1]. Other hypotheses relate biphasic morphine actions to shifting levels of brain neurotransmitters, such as acetylcholine [5] or dopamine [3, 6, 13]. And, several behavioral theories might explain the biphasic time-effect pattern in terms of underlying opponent processes or compensatory responses [11, 12, 14]. According to these formulations, experience with the drug would be expected to produce tolerance to the primary effect (e.g., hypoactivity) as it strengthens the secondary effect (e.g., hyperactivity). The present data provide no basis for choosing among these particular alternatives; nevertheless, one hypothesis does appear to be weakened by the results of Experiment 2. This hypothesis states that stimulant and depressant effects are the result of a single dose-related mechanism: Stimulant effects occur at low doses and depressant effects occur at high doses. Accordingly [15], "the late stimulant actions observed with higher doses of morphine could be due to a decrease in the brain level of drug to a concentration equal to that achieved with a low dose initially" (p. 856). The validity of this hypothesis rests upon the assumption that low doses of the drug are initially purely excitatory. However, the results of Experiment 2 indicate that even very low doses of morphine have mixed actions, suggesting that, at least in the hamster, biphasic effects of morphine are not the result of a single dose related mechanism.

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