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BRIEF COMMUNICATION

Environment-Specific Cross-Sensitization Between the Locomotor Activating Effects of Morphine and Amphetamine

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VEZINA, P., A. A. GIOVINO, R. A. WISE AND J. STEWART. *Environment-specific cross-sensitization between the locomotor activating effects of morphine and amphetamine.* PHARMACOL BIOCHEM BEHAV 32(2) 581–584, 1989.— Groups of eight rats each were preexposed on four occasions to 10 or 20 mg/kg morphine sulfate, IP, either in activity boxes where activity was measured for two hours (COND, conditioning groups) or in their home cages (UNPAIRED groups). On alternate days these groups were administered saline in the other environment. Two groups of eight rats each served as CONTROL groups (one for each preexposure dose) and were administered saline in both environments. On the day following morphine preexposure, all animals were administered 0.5 mg/kg d-amphetamine sulfate, IP, prior to being tested in the activity boxes. On this test, the COND group preexposed to 10 mg/kg morphine showed higher levels of activity than either of its respective UNPAIRED or CONTROL groups. The COND group preexposed to 20 mg/kg morphine was significantly more active than its UNPAIRED group, but not more active than its CONTROL group. The implications of such environment-specific cross-sensitization between the activity effects of opiate and stimulant drugs are discussed.

Environment-specificity Cross-sensitization Locomotor activity Morphine Amphetamine Conditioning

THERE have been numerous reports of sensitization to the locomotor activating effects of morphine and other opiates when these drugs are administered repeatedly either systemically (1, 4, 12, 14, 15, 22) or directly into the ventral tegmental area (VTA), site of the cell bodies of the mesolimbic dopamine neurons (8, 11, 23). Similarly, numerous studies have also shown that the repeated systemic administration of amphetamine leads to the sensitization of its locomotor activating effects [for a review, see (16)].

Considerable evidence now suggests that activity in the mesolimbic dopamine system underlies the locomotor activating effects of these two classes of drugs and that modifications in the functioning of this system are responsible for sensitization to morphine and other opioids [(see (9)] and to amphetamine and other stimulants [(see (16)]. Thus cross-sensitization was recently demonstrated between the locomotor activating effects of amphetamine and those of morphine administered either systemically or into the VTA (19). Further, the sensitized response to morphine was specific to the environment where amphetamine had been previously administered. That is, animals preexposed to amphetamine elsewhere were no more active than saline control group animals when given morphine in the test environment. These findings of environment-specific cross-sensitization extended earlier findings with amphetamine (21) and morphine (13,23) demonstrating that, if deliberate care is taken to pair drug exposure exclusively with a distinctive set of environmental stimuli, the expression of sensitization can come under strong stimulus control.

Cross-sensitization to systemic amphetamine has also been reported following the development of sensitization to intra-VTA enkephalin (9), but without testing for environment-specificity. Here, we report environment-specific cross-sensitization in animals preexposed to systemic injections of morphine and tested with systemic amphetamine.

METHOD

Subjects

Forty-eight male Wistar rats (Charles River, Canada), weighing 250–300 g on arrival, were used. They were housed individually with food and water freely available in a 12-hr

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dark/12-hr light reverse cycle room. All testing was done during the dark cycle.

Apparatus and Materials

A bank of 12 activity boxes [described elsewhere, (19)] was used to measure locomotor activity. Horizontal locomotor activity was estimated by two photocells positioned along the longitudinal axis of each box. Activity counts were automatically recorded and totalled by computer each hour for each animal during the course of the session. The boxes were in a room lit dimly with red light. White noise was continuously present to mask extraneous noise.

Morphine sulfate (B.D.H. Chemicals, Toronto) and d-amphetamine sulfate (Smith, Kline and French, Canada) were prepared in physiological saline and injected IP in a 1.0 ml/kg volume. Saline injections were made in the same volume by the same route.

Design and Procedure

The study was conducted in two replications; the design and procedures used in each was identical. In the first, the dose of morphine to which animals were preexposed was 10 mg/kg. In the second, it was 20 mg/kg. In each replication, three groups of eight animals each were used.

Morphine preexposure phase. The preexposure phase consisted of four 2-day blocks. On the first day of each block, animals received their injections prior to being placed in the activity boxes for 2 hr. On the following day, animals were injected in the animal room and immediately returned to their home cages. Animals in the conditioning groups (COND) received either 10 or 20 mg/kg morphine prior to being placed in the activity boxes and saline injections in their home cages. UNPAIRED group animals were administered saline in the activity boxes and their assigned morphine injection in their home cages. The CONTROL group animals received saline injections in both environments.

Amphetamine test day. On the day following the preexposure phase, all animals were injected with 0.5 mg/kg amphetamine and tested in the activity boxes for 2 hr.

RESULTS

Morphine Preexposure Phase

The mean activity counts obtained on Days 1 and 4 of the morphine preexposure phase for the 10 mg/kg (top panel) and the 20 mg/kg (bottom panel) replications are shown in Fig. 1. Separate groups × days × hours analyses of variance (ANOVA's) were conducted on these data for each replication.

It can be seen that in the 10 mg/kg experiment, animals that received morphine in the activity boxes (Group COND) were more active than animals in the other two groups on both days, a finding reflected in the significant groups effect, $F(2,21)=22.26$, $p<0.001$. The finding that activity levels in Group COND increased in the second hour while they decreased in the remaining two groups is reflected in the significant groups × hours interaction, $F(2,21)=15.59$, $p<0.001$. Animals in Group COND were slightly more active on Day 4 than on Day 1 although not significantly so.

In the 20 mg/kg experiment, differences between groups were reflected primarily in the significant groups × days, $F(2,21)=18.23$, $p<0.001$, and groups × hours, $F(2,21)=60.34$, $p<0.001$, interactions. It can be seen from Fig. 1 that animals that received morphine in the activity boxes (Group COND) had greatly suppressed activity levels in the first hour of the first day. By the fourth day of preexposure to morphine, these animals showed much reduced suppression to morphine although their activity levels remained slightly lower than those of CONTROL group animals in the first hour. In the second hour of this day, animals in Group COND were clearly more active than those in the other two groups and more active than they had been in the second hour of Day 1. Again, activity levels in Group COND increased in the second hour while they decreased in the other two groups.

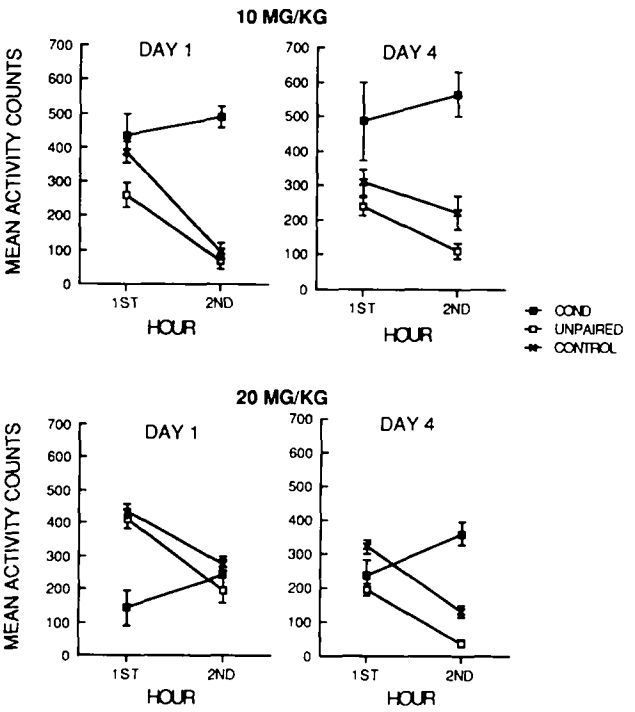

FIG. 1. Mean activity counts (±1 S.E.M.) obtained on Days 1 and 4 of the morphine preexposure phase for each of the three groups in the 10 mg/kg (top panel) and the 20 mg/kg (bottom panel) replications.

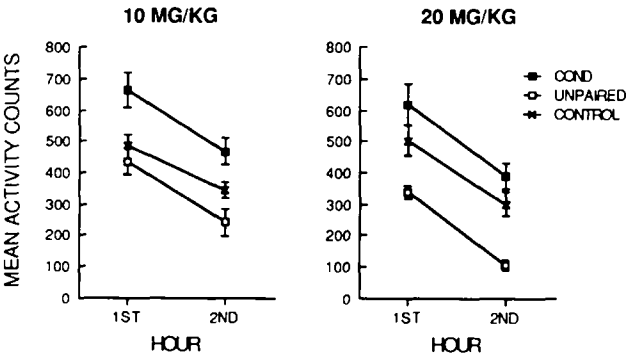

FIG. 2. Mean activity counts (±1 S.E.M.) obtained on the amphetamine test day for each of the three groups in the 10 mg/kg (left panel) and the 20 mg/kg (right panel) replications.

Amphetamine Test Day

The data from this day, when all groups were tested with amphetamine, are shown in Fig. 2. Separate ANOVA's (groups × hours) were conducted on the data from each replication. At 10 mg/kg, both the groups, $F(2,21)=11.25$, $p<0.001$, and the hours, $F(1,21)=44.88$, $p<0.001$, effects were significant. Post hoc Scheffé comparisons revealed that Group COND was significantly more active than both the UNPAIRED ($p<0.001$) and the CONTROL ($p<0.05$) groups which did not differ significantly from each other. All groups were less active in the second hour under amphetamine. At 20 mg/kg, again both the groups, $F(2,21)=16.46$, $p<0.001$, and the hours, $F(1,21)=79.25$, $p<0.001$, effects were significant. Scheffé comparisons revealed that Groups COND and CONTROL were significantly more active than Group UNPAIRED (p's<0.001 and 0.01, respectively) but that, although Group COND was slightly more active than the CONTROL group, the difference, in this case, was not significant.

DISCUSSION

In these experiments, we have demonstrated that preexposure to morphine affects the subsequent response to a systemic injection of amphetamine given in the same environment. These findings extend earlier findings of cross-sensitization to systemic amphetamine following preexposure to intra-VTA enkephalin (9) and support the view that modifications in a common system (the mesolimbic dopamine system) are responsible for sensitization to these two classes of drugs (9,16). In the present experiments, however, not only was cross-sensitization obtained but it was found to be environment-specific. That is, cross-sensitization to amphetamine was evident only in those animals tested in the environment where they had previously received morphine, demonstrating, once again, that the expression of sensitization can come under strong stimulus control [see also (13, 19, 21, 23)]. Interestingly, observation of the time course of locomotor activity produced in the COND groups by each of the two drugs reveals that activity increased from the first to the second hour with morphine but decreased with amphetamine. Thus, even though animals in the COND groups were more active than animals in the other groups when given amphetamine, the time course of locomotor activity was characteristic of the drug amphetamine, not of morphine. This would suggest that the conditioning environment acted to modulate the expression of the drug action. In the case when the activity box environment had been repeatedly paired with morphine (and thus became a CS+), a sensitized response to amphetamine was seen in the COND groups. Conversely, in the case of the UNPAIRED groups, when the activity box environment was repeatedly paired with the absence of morphine (and thus became a CS−), a selective depression of amphetamine-induced activity was seen.

Surprisingly, the locomotor activity induced by amphetamine in the COND group preexposed to 20 mg/kg morphine, although higher, was not significantly higher than that induced in its respective CONTROL group and was actually lower than that induced in the COND group preexposed to 10 mg/kg morphine (see Fig. 2). Given the view that the development of tolerance to the depressant effects and sensitization to the excitatory effects of morphine on locomotor activity are mediated via actions on separate neuronal systems (2, 3, 17, 22) and that the dose of amphetamine used produces only stimulant effects on this behavior, it had been expected that preexposure to the higher dose of morphine would produce greater cross-sensitization to amphetamine even with the depressant effects of this dose still partly in evidence on the last day of preexposure (see Fig. 1, bottom panel). It may be, however, that even though the development of sensitization and tolerance may represent changes occurring in two separate neuronal systems, each of these systems may influence the changes occurring in the other, especially when morphine is administered systemically. For example, selective lesions of dopamine neurons in the VTA, where morphine produces only increased locomotor activity which shows sensitization with repeated injections (8,23), have been shown to delay the development of tolerance to morphine catalepsy (7). Conversely, the development or lack of development of tolerance to the depressant effects of high doses of morphine [possibly by changes occurring in the nucleus raphe pontis (5), or other brain regions (6,10)] may influence the development of sensitization to the excitatory effects of morphine. Interestingly, sensitization has been found *not* to develop when low systemic doses of morphine, which elicit only increases in activity, are used (1). In the present experiments, incomplete tolerance to the depressant effects of the 20 mg/kg dose of morphine during preexposure (as evidenced, in Fig. 1, by the lower activity levels of Group COND relative to Group CONTROL in the first hour of Day 4, bottom panel, and the overall lower activity levels on this day of this COND group, bottom panel, compared to those of the COND group preexposed to the 10 mg/kg dose of morphine, top panel) may have attenuated the development of sensitization to the excitatory effects of morphine and thus cross-sensitization to amphetamine. The lower activity levels of the UNPAIRED group in the 20 mg/kg replication on the amphetamine test day may thus reflect the enhanced inhibition by a CS− of a not fully sensitized response to amphetamine [see (20)].

During morphine preexposure, animals in the COND groups received their morphine injections on the first day of each block while those in the UNPAIRED groups received them on the second. As a result, animals in the COND groups were tested with amphetamine 48 hours after the last morphine injection while those in the UNPAIRED groups were tested 24 hours after the last injection. This raised the possibility that the amphetamine test results were biased especially in the 20 mg/kg replication in which residual depressant effects of this dose of morphine may still have been present in UNPAIRED group animals and lowered the activity levels of these animals on this test. This possibility is unlikely, however. First, observation of the activity levels of group COND on Day 4 of the 20 mg/kg replication reveals that, although some depression was still evident in the first hour, none was evident in the second making it unlikely that depressant effects sufficient to inhibit the amphetamine response were present on the following day. Second, animals in this replication were given another amphetamine test three days after the morphine preexposure phase. The findings obtained on this test (data not shown) were similar to those obtained on the first again making it unlikely that differences between groups COND and UNPAIRED in the presence of residual effects of morphine on the amphetamine test day may have biased the results.

As noted earlier, the present results support the view that changes in the mesolimbic dopamine system are responsible for sensitization to the locomotor activating effects of mor-

phine and amphetamine. If it is considered that this neural system has also been implicated in the mediation of the rewarding properties of these drugs (24), the present findings lend further support to the view that such drugs may be more effective in the instigation not only of their behavioral activating but also of their rewarding effects when administered in an environment where they were previously experienced (18).

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