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Acute Morphine Dependence in the Hamster[1]

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SCHNUR, P. *Acute morphine dependence in the hamster*. PHARMACOL BIOCHEM BEHAV **38**(4) 711–713, 1991.—Two experiments investigated naloxone-precipitated withdrawal following a brief course of morphine administration in hamsters. In Experiment 1, observable withdrawal symptoms (e.g., wet-dog shakes) were elicited by two doses of naloxone (0.4 and 1.0 mg/kg) following four and eight daily injections of morphine (15 mg/kg), a regimen that replicated previous studies in our laboratory using a locomotor activity paradigm. At the lower dose of naloxone, the frequency of withdrawal signs was greater after eight than after four morphine injections. In Experiment 2, observable withdrawal symptoms were elicited by the same two doses of naloxone, 70 min after a single morphine injection. These results suggest that acute dependence in the hamster, as in other species, begins to develop with the first morphine exposure.

Acute dependence Withdrawal Naloxone Morphine Hamster

PREVIOUS work in our laboratory has demonstrated that morphine has a biphasic effect on running wheel activity in the hamster. At low doses (<5 mg/kg), morphine elicits hyperactivity, whereas at higher doses, morphine elicits hypoactivity followed by hyperactivity, the magnitude and duration of the hypoactivity being dose related (20–22). Moreover, when naloxone or naltrexone is given during the period of hyperactivity, hyperactivity is replaced by hypoactivity (19, 23, 24). We have argued that such naloxone-precipitated hypoactivity in morphine-treated animals, but not in saline-treated controls, reveals the existence of an underlying morphine-induced process. The purpose of the present experiments was to demonstrate that such naloxone-precipitated hypoactivity is a manifestation of opiate withdrawal induced by acute dependence on morphine.

Acute dependence refers to the precipitation of withdrawal symptoms following a single or a small number of drug administrations (2, 3, 5–8, 10–14, 16, 17, 25). In our work with hamsters, typically, we have given the naloxone (0.4 mg/kg) challenge after four subcutaneous (SC) injections of morphine (15 mg/kg). Under this regimen, naloxone-induced hypoactivity is reliable and dose-related (24). The present study was designed to investigate whether the same dosing regimen of morphine and naloxone as that used in the activity wheel paradigm would elicit observable withdrawal symptoms. Thus, in Experiment 1, animals were challenged with naloxone (0, 0.4 or 1 mg/kg) after 4 and again after 8 daily morphine administrations and observed for signs of opiate withdrawal such as wet dog shakes, teeth chattering, etc. In Experiment 2, animals were observed for signs of naloxone-precipitated withdrawal after a single injection of morphine.

METHOD

Subjects, Apparatus and Materials

The subjects were 48 female golden Syrian hamsters with a mean weight of 130 grams. They were housed singly in stainless steel cages with free access to Purina lab chow and water in a temperature- and humidity-controlled vivarium. Animals were observed in a transparent plastic cage (45.7 × 24.1 × 20.3 cm). Morphine sulfate and naloxone hydrochloride were dissolved in saline and given subcutaneously in the dorsal surface of the neck in 1 ml/kg volumes. All doses refer to the salt.

Procedure

Experiment 1. Animals were divided randomly into two groups (n = 12). On Days 1–3, animals were removed from the home cage, given an injection of either morphine (Group M) or saline (Group S) and replaced in the home cage immediately after the injection. On Day 4, animals were given either morphine or saline as on Days 1–3. One hour later, they were removed from the home cage and placed in the plastic observation cage. After a 10-min period of observation, four animals chosen randomly from each group were given an injection of one of three doses of naloxone [0 (saline), 0.4, 1.0 mg/kg] and replaced in the plastic cage for a 30-min observation period. The frequency of opiate withdrawal signs was noted: Wet-dog shakes, paw shakes, teeth chattering, abdominal writhing, yawning and stools. On Days 5–7, the treatments of Days 1–3 were repeated and, on Day 8, a second test, identical to that on Day 4 was conducted.

Experiment 2. The procedures of this experiment were conducted in a single test day. Twenty-four animals were assigned randomly to six treatments (n = 4) created by the factorial combination of injection 1 (morphine or saline) and injection 2 (three doses of naloxone). On the test day, animals were removed from the home cage, given an injection of either morphine (Group M) or saline (Group S) and returned immediately to the home cage. One hour later, they were removed from the home cage and placed in the plastic observation cage. After a 10-min observation pe-

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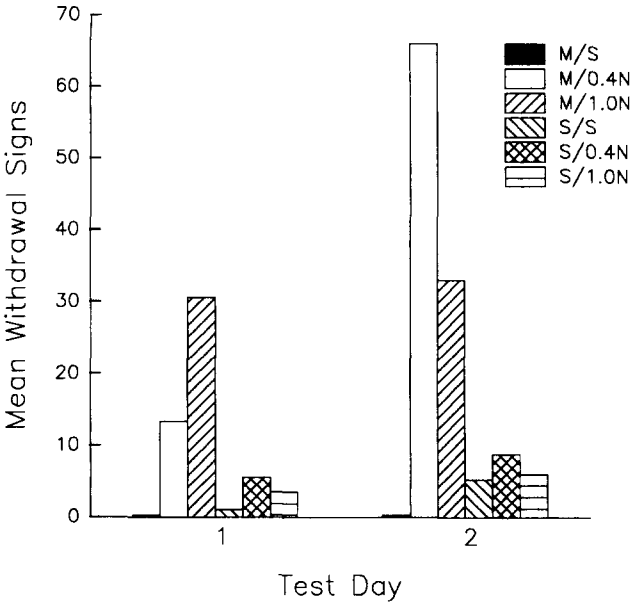

FIG. 1. Mean number of withdrawal signs on Test Days 1 and 2 for groups receiving morphine or saline followed by one of three doses of naloxone (0, 0.4, 1.0 mg/kg) in Experiment 1.

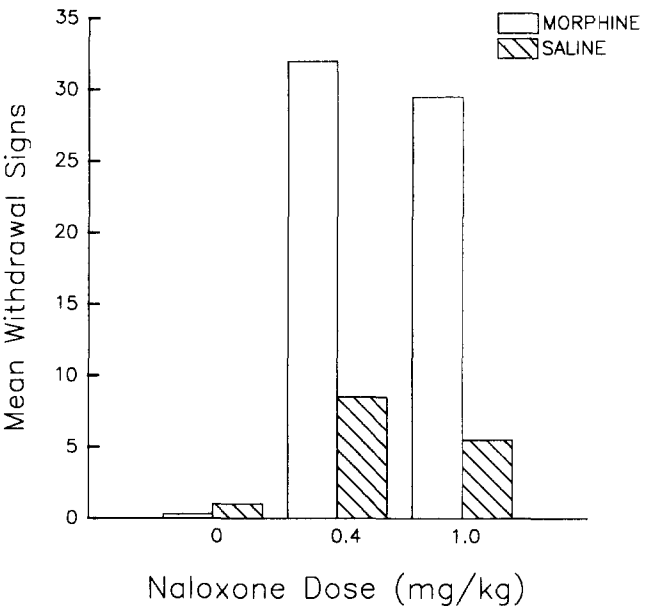

FIG. 2. Mean number of withdrawal signs as a function of naloxone dose (0, 0.4, 1.0 mg/kg) for animals receiving morphine or saline in Experiment 2.

riod, animals were given an injection of one of three doses of naloxone [0 (saline), 0.4, 1.0 mg/kg] and replaced in the plastic cage for a 30-min observation period. Withdrawal behaviors were scored as in Experiment 1.

An alpha level of $p<0.05$ was adopted for all statistical tests.

RESULTS AND DISCUSSION

The mean number of withdrawal signs during the 30-min observation period on Test Days 1 and 2 in Experiment 1 is shown in Fig. 1. It is clear that naloxone, at doses of 0.4 and 1 mg/kg, precipitated withdrawal symptoms in morphine-treated animals on both test days. The frequency of withdrawal signs in morphine-treated animals challenged with saline and in saline controls was low. Naloxone-precipitated withdrawal increased with exposure to morphine, although this effect was more pronounced at the lower than at the higher naloxone dose. A $2\times3\times2$ (Morphine $\times$ Naloxone $\times$ Test Day) mixed factorial analysis of variance (ANOVA) indicated that the effects of morphine, $F(1,18)=13.32$, naloxone, $F(2,18)=6.47$, and the interaction, $F(2,18)=4.57$, were all significant. In addition, the effect of days, $F(1,18)=5.59$, was significant. The Naloxone $\times$ Days, $F(2,18)=3.47$, and the Morphine $\times$ Naloxone $\times$ Days, $F(2,18)=3.47$, interactions were not significant. Fisher's LSD test indicated that withdrawal signs were more frequent in Group M/0.4N and Group M/1.0N than in Group M/S and that there were more withdrawal signs on the second test day than on the first in Group M/0.4N.

Figure 2 shows the mean number of withdrawal signs during the 30-min observation period in Experiment 2. Although the frequency of withdrawal was lower than in the first experiment, it is evident that both doses of naloxone precipitated withdrawal symptoms in animals previously injected with morphine. As in Experiment 1, the frequency of withdrawal signs in morphine-treated animals challenged with saline and in saline controls challenged with naloxone was low. A 2×3 (Morphine $\times$ Naloxone) factorial ANOVA indicated that the effects of morphine, $F(1,18)=7.97$, and of naloxone, $F(2,18)=5.06$, were significant. The interaction was not significant. Fisher's LSD test indicated that animals given morphine followed by either dose of naloxone (Groups M/0.4N and M/1.0N) displayed more withdrawal symptoms than either animals given morphine followed by saline (Group M/S) or animals given saline followed by naloxone (Groups S/0.4N and S/1.0N)

The present results indicate that a naloxone challenge elicits observable withdrawal signs among hamsters briefly exposed to morphine. In Experiment 1, withdrawal signs were elicited by naloxone following the fourth daily injection of morphine. In Experiment 2, withdrawal signs were elicited by naloxone after only a single injection of a moderate dose of morphine. These findings suggest that, in the hamster, the development of physical dependence, as measured by naloxone-precipitated withdrawal, begins with the first opiate administration. Similar effects of naloxone have been reported after the short-term administration of opiates in a variety of species including humans (1, 7, 8), rats (4,15), dogs (10, 12, 13), monkeys (11), mice (9,18) and gerbils (17).

In both experiments, withdrawal signs appeared within 5 min of the naloxone injection, reached peak intensity at approximately 20 min postinjection and diminished after 30 min. Since the postinjection observation period was only 30 min, however, we cannot be precise about the duration of the precipitated abstinence syndrome. The precipitated abstinence syndrome in the hamster consists of several signs, as defined above. The relative contribution of these signs to the abstinence syndrome, in terms of frequency of occurrence, was (in rank order): Wet-dog shakes/paw shakes, teeth chattering, writhing, yawning, defecation. Comparable results have been noted in rats, mice and gerbils (17).

Finally, since the drug administration schedule employed in Experiment 1 replicated that used in the activity wheel paradigm, it is likely that naloxone-precipitated hypoactivity among morphine-treated hamsters in our earlier studies [e.g., (24)] was caused by the incompatability of running with manifest withdrawal signs. The similar time course of naloxone-precipitated abstinence

signs in the present experiments and of naloxone-precipitated hypoactivity in the running wheel experiments (19,24) is consistent with that suggestion. The running wheel activity paradigm might provide a convenient, sensitive and reliable alternative to behavioral observations for quantifying naloxone-precipitated opiate withdrawal in the hamster.

REFERENCES

1. Bickel, W. K.; Stitzer, M. L.; Liebson, I. A.; Bigelow, G. E. Acute physical dependence in man: Effects of naloxone after brief morphine exposure. J. Pharmacol. Exp. Ther. 244:126–132; 1988.
2. Cheney, D. L.; Goldstein, A. Tolerance to opioid narcotics: Time course and reversibility of physical dependence in mice. Nature 232: 477–478; 1971.
3. Eisenberg, R. M. Further studies on the acute dependence produced by morphine in opiate naive rats. Life Sci. 31:1531–1540; 1982.
4. Eisenberg, R. M.; Sparber, S. B. Changes in plasma corticosterone levels as a measure of acute dependence upon levorphanol in rats. J. Pharmacol. Exp. Ther. 211:364–369; 1979.
5. Gellert, V. F.; Sparber, S. B. A comparison of the effects of naloxone upon body weight loss and suppression of fixed ratio operant behavior in morphine-dependent rats. J. Pharmacol. Exp. Ther. 201: 44–54; 1977.
6. Grilly, D. M.; Gowans, G. C. Acute morphine dependence: Effects observed in shock and light discrimination tasks. Psychopharmacology (Berlin) 88:500–504; 1986.
7. Heishman, S. J.; Stitzer, M. L.; Bigelow, G. E.; Liebson, I. A. Acute opioid physical dependence in humans: Effect of varying the morphine-naloxone interval. I. J. Pharmacol. Exp. Ther. 250:485–491; 1989.
8. Heishman, S. J.; Stitzer, M. L.; Bigelow, G. E.; Liebson, I. A. Acute opioid physical dependence in postaddict humans: Naloxone dose effects after brief morphine exposure. J. Pharmacol. Exp. Ther. 248:127–134; 1989.
9. Jacob, J. J.; Barthelemy, C. D.; Tremblay, E. C.; Colombel, M. L. Potential usefulness of single-dose acute physical dependence on and tolerance to morphine for the evaluation of narcotic antagonists. In: Braude, M. C.; Harris, L. S.; May, E. L.; Smith, J. P.; Villareal, J. E., eds. Advances in biochemical psychopharmacology, narcotic antagonists, vol. 8. New York: Raven; 1974:299–318.
10. Jacob, J. J.; Michaud, G. M. Acute physical dependence in the waking dog after a single low dose of morphine. Psychol. Med. 4:270–273; 1974.
11. Krystal, J. H.; Redmond, D. E. A preliminary description of acute physical dependence on morphine in the vervet monkey. Pharmacol. Biochem. Behav. 18:289–291; 1983.
12. Martin, W. R.; Eades, C. G. Demonstration of tolerance and physical dependence in the dog following a short-term infusion of morphine. J. Pharmacol. Exp. Ther. 133:262–270; 1961.
13. Martin, W. R.; Eades, C. G. A comparison between acute and chronic physical dependence in the chronic spinal dog. J. Pharmacol. Exp. Ther. 146:385–394; 1964.
14. Martin W. R.; Sloan, J. W. Neuropharmacology and neurochemistry of subjective effects, analgesia, tolerance, and dependence produced by narcotic analgesics. In: Martin, W. R., ed. Handbook of experimental pharmacology, drug addiction I, vol. 45. Berlin: Springer-Verlag; 1977:43–158.
15. Meyer, D. R.; Sparber, S. B. Evidence of possible opiate dependence during the behavioral depressant action of a single dose of morphine. Life Sci. 21:1087–1094; 1977.
16. Pilcher, C. W. T. Inducing dependence by a single administration of morphine in rats. Br. J. Pharmacol. 57:430–431; 1976.
17. Ramabadran, K. Naloxone-precipitated abstinence in mice, rats and gerbils acutely dependent on morphine. Life Sci. 33(Suppl. I):385–388; 1983.
18. Ritzmann, R. F. Opiate dependence following acute injections of morphine and naloxone: The assessment of various withdrawal signs. Pharmacol. Biochem. Behav. 14:575–577; 1981.
19. Schnur, P. Effects of naloxone and naltrexone on morphine elicited changes in hamster locomotor activity. Physiol. Psychol. 13:26–32; 1985.
20. Schnur, P. Morphine tolerance and sensitization in the hamster. Pharmacol. Biochem. Behav. 22:157–158; 1985.
21. Schnur, P.; Bravo, F.; Trujillo, M. Tolerance and sensitization to the effects of low doses of morphine in the hamster. Pharmacol. Biochem. Behav. 19:435–439; 1983.
22. Schnur, P.; Bravo F.; Trujillo, M.; Rocha, S. Biphasic effects of morphine on locomotor activity in the hamster. Pharmacol. Biochem. Behav. 18:367–371; 1983.
23. Schnur, P.; Hang, D. Naloxone reversal of morphine elicited hyperactivity. Life Sci. 40:329–333; 1987.
24. Schnur, P.; Raigoza, V. P. Effects of naloxone on morphine induced sedation and hyperactivity in the hamster. Pharmacol. Biochem. Behav. 24:849–856; 1986.
25. Stevens, D. R.; Klemm, W. R. Morphine-naloxone interactions: A role for nonspecific morphine excitatory effects in withdrawal. Science 205:1379–1380; 1979.