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Independent Effects of Morphine and Apomorphine on Stereotyped Gnawing in the Hamster

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SCHNUR, P. AND R. A. MARTINEZ. *Independent effects of morphine and apomorphine on stereotyped gnawing in the hamster.* PHARMACOL BIOCHEM BEHAV **32**(3) 589–594, 1989.—Eight experiments were conducted to investigate the effects of apomorphine, a dopaminergic agonist, and of morphine, an opiate, on stereotyped behavior in the hamster. Animals were observed at two minute intervals for one hour and incidents of stereotyped gnawing, licking and sniffing were recorded using a time-sampling method. Both morphine and apomorphine produced dose-related increases in stereotyped gnawing. A low dose of the opiate antagonist, naloxone (0.4 mg/kg), blocked morphine-induced gnawing but neither that dose nor higher doses of naloxone (1, 4 and 10 mg/kg) blocked apomorphine-induced gnawing. A low dose of the dopaminergic antagonist, haloperidol (0.05 mg/kg), blocked apomorphine-induced gnawing but did not block morphine-induced gnawing. Further experiments indicated that morphine administration did not sensitize, or influence in any way, subsequent apomorphine-induced stereotyped behavior.

Morphine Apomorphine Stereotyped behavior Hamsters Naloxone Haloperidol

INTERACTIVE influences on stereotyped behaviors between opiate agonists and antagonists on the one hand, and dopaminergic agonists and antagonists on the other, have been investigated in rats (1, 3–5, 7, 8, 10, 12–14, 17), guinea pigs (5) and mice (11). Several studies have found that morphine pretreatment potentiates apomorphine-induced stereotyped behavior (3–5, 10–12, 14), and that the opiate antagonist, naloxone, inhibits apomorphine-induced stereotyped behavior (4, 5, 8, 17). A number of studies, however, have reported the opposite results, with morphine antagonizing apomorphine-induced stereotyped behavior (4,13) and naloxone potentiating it (13). In one study (4), a high dose of morphine, in the form of a 75 mg pellet implanted for three days, potentiated the effects of apomorphine, whereas a low dose, in the form of one 15 mg/kg injection, antagonized the effects of apomorphine. The purpose of the present experiments was to examine the independent and interactive effects of morphine and apomorphine on stereotyped behaviors in the hamster. In addition, the present experiments investigated the effects of naloxone, and haloperidol, a dopaminergic antagonist, on both morphine- and apomorphine-induced stereotyped behaviors.

Eight experiments are described. The first experiment established the dose-effect and time-effect functions for apomorphine-induced stereotyped behaviors in the hamster. The next three experiments were designed to investigate whether morphine pretreatment would potentiate apomorphine-induced stereotyped gnawing in the hamster. Experiment 2 tested the effects of a single dose of morphine on gnawing elicited by several doses of apomorphine. Since the effects of morphine in the hamster are dose-dependent (15,16), Experiment 3 tested the effects of several doses of morphine on gnawing induced by a marginally effective dose of apomorphine. If morphine were to effectively potentiate the effects of apomorphine, it should be evident at this dose of apomorphine. Since the effects of morphine in the hamster also are time-dependent (15,16), Experiment 4 tested the effects of a single dose of morphine administered at different times prior to an injection of apomorphine. The next three experiments tested the effects of naloxone on morphine- and apomorphine-induced gnawing in the hamster. Experiment 5 investigated the effects of a single dose of naloxone on gnawing elicited by several doses of morphine. Experiment 6 tested the effects of that same dose of naloxone on gnawing elicited by apomorphine. In an attempt to determine whether higher doses than that used in Experiment 6 would block apomorphine-induced gnawing, Experiment 7 tested the effects of five doses of naloxone on apomorphine-induced gnawing. Finally, Experiment 8 investigated the effects of haloperidol on morphine- and apomorphine-induced gnawing.

METHOD

Subjects

Eighty female hamsters (*Mesocricetus auratus*) with a mean weight of 141 g were used. They were obtained from Sasco, Inc. (Omaha, NE), housed individually in hanging wire mesh cages, and maintained on a 12:12 hr light-dark cycle (lights on at 0700). Hamsters were given free access to tap water, Purina lab chow and paper nesting materials.

Apparatus and Materials

The apparatus consisted of eight chambers (Ralph Gerbrands, Model C) in which animals were placed for observation. Each chamber (20×23×19 cm) had a Plexiglas ceiling and two Plexiglas side walls, aluminum end walls and a grid floor. On one end wall were a response lever, a lamp fixture and a recessed food cup.

The following drugs were used: morphine sulfate solution (Lilly), naloxone hydrochloride (Dupont, Endo), apomorphine hydrochloride (Sigma), haloperidol lactate solution (McNeil). Morphine, naloxone and apomorphine were diluted as needed with bacteriostatic saline. Haloperidol was diluted with deionized water. All injections were given SC in the dorsal surface of the neck in 1 ml/kg volumes, except where otherwise noted.

Procedure

Eight experiments using the same general procedures were completed. Where animals served in more than one experiment, at least one week elapsed between experimental procedures. In all experiments, animals were assigned randomly to treatments. In each experiment, animals were given either a single injection or a series of two injections (10 min apart, unless otherwise specified) and then placed in the chambers for one hour. Animals were observed at 0.6 sec tone-cued 2 min intervals by an observer unaware of the drug treatments and behavior was classified as either gnawing, licking, sniffing or other. Gnawing was defined as persistent chewing on objects in the box (typically the grid floor, lever and lamp fixture). Licking was defined as observable movements of the tongue on the cage surface (typically the walls). Sniffing was defined as stimulus-directed movements of the nares (typically with the snout placed between the bars of the grid floor). Using this scoring procedure, the frequency of observed behaviors ranged from 0 to 30 during the 1 hr period. For graphic presentation, frequency (f) was converted to percentage (f/30 × 100).

Experiment 1. The purpose of the first experiment was to determine the dose-response function for apomorphine-induced stereotyped behavior in the hamster. Hamsters were given apomorphine doses (mg/kg) of either 0 (saline, n=8), 0.01 (n=4), 0.1 (n=8), 1 (n=8) or 10 (n=4). All doses here and below are expressed as the salt.

Experiments 2, 3 and 4. The purpose of these experiments was to test the effects of acute morphine administration on apomorphine-induced gnawing. Experiments 2 and 3 used the same 32 hamsters as used in Experiment 1, while Experiment 4 used 16 experimentally naive hamsters. In Experiment 2, animals received a series of two injections, 10 min apart, before being observed for 1 hr. The first injection consisted of either 0 or 15 mg/kg of morphine. The second injection consisted of either 0, 0.01, 0.1 or 1 mg/kg of apomorphine. Thus, 8 groups (n=4) were formed by the factorial combination of the two treatments. In Experiment 3, the first injection consisted of 0, 2.5, 5 or 15 mg/kg of morphine and the second injection consisted of 0 of 0.1 mg/kg of apomorphine. In Experiment 4, the two injections were either 10, 30 or 60 min apart. Each hamster was tested once at each interinjection interval in a counterbalanced design. The first injection was either 0 or 15 mg/kg of morphine and the second injection was either 0 or 0.1 mg/kg of apomorphine.

Experiments 5, 6 and 7. Experiments 5, 6 and 7 tested the effects of naloxone on morphine- and apomorphine-induced gnawing. In each experiment, animals received two injections 10 min apart before being observed for 1 hr. The same 32 hamsters were used in these experiments as in Experiment 1. In Experiment 5, the first injection consisted of either 0 or 0.4 mg/kg of naloxone and the second injection consisted of 0, 5, 15 and 30 mg/kg of morphine. The 30 mg/kg dose was administered in a 2 ml/kg volume. In Experiment 6, the first injection consisted of either 0 or 0.4 mg/kg of naloxone and the second injection consisted of either 0 or 1 mg/kg of apomorphine. In Experiment 7, hamsters served in a repeated measures design. On five successive days, the first injection was one of five randomly determined doses of naloxone (0, 0.4, 1, 4 or 10 mg/kg). For half of the animals, the second injection was saline on each day; for the other half, it was apomorphine (1 mg/kg) on each day.

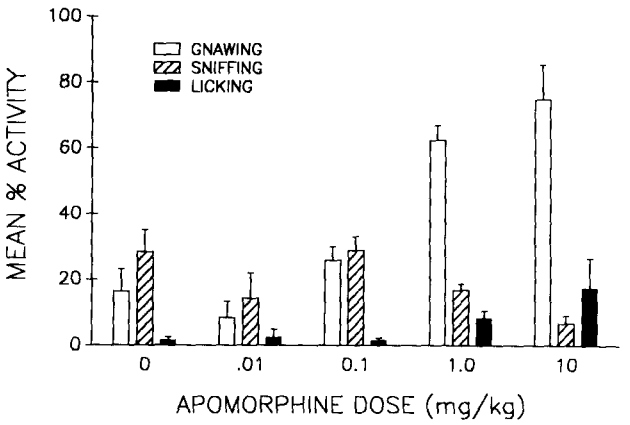

FIG. 1. Mean (+S.E.M.) percent of gnawing, sniffing and licking as a function of apomorphine dose in Experiment 1. For doses of 0, 0.1 and 1 mg/kg, n=8; for doses of 0.01 and 10 mg/kg, n=4.

Experiment 8. The purpose of Experiment 8 was to test the effects of haloperidol on morphine- and apomorphine-induced gnawing. Two injections, 20 min apart, were given to 32 experimentally naive hamsters. The first injection was either a water vehicle or haloperidol (0.05 mg/kg) and the second injection was either saline, apomorphine (1 mg/kg) or morphine (30 mg/2 ml/kg).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) techniques supplemented by *t*-tests for individual comparisons (9). A 0.05 level of significance was adopted for all statistical comparisons.

RESULTS

Figure 1 shows the mean percent of gnawing, licking and sniffing in Experiment 1 as a function of apomorphine dose. It is evident that at the three lowest doses, sniffing predominated. As dose increased, however, gnawing increased so that at the 1 and 10 mg/kg doses, gnawing predominated, occurring in 63% and 75% of the observation periods, respectively. One-way ANOVAs on the gnawing, licking and sniffing data indicated that the effect of dose was significant for each response measure, $F(4,27)=21.44$, $F(4,27)=5.34$ and $F(4,27)=3.07$, respectively. Subsequent *t*-tests indicated that the 1 mg/kg dose, $t(27)=6.08$, and the 10 mg/kg dose,

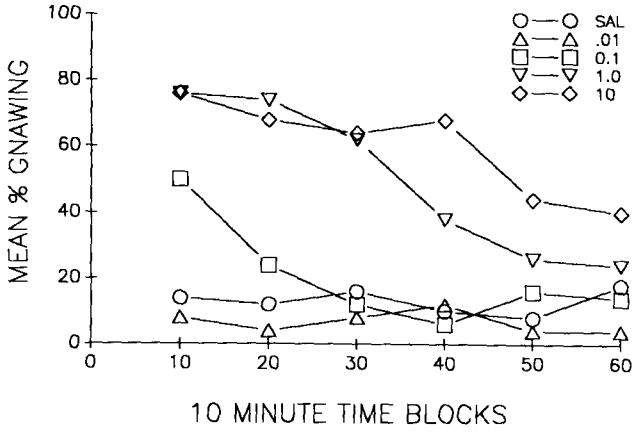

FIG. 2. Mean percent of gnawing as a function of 10 min time blocks for each dose of apomorphine in Experiment 1. For doses of 0, 0.1 and 1 mg/kg, n=8; for doses of 0.01 and 10 mg/kg, n=4.

$t(27)=6.32$, elicited significantly more gnawing than did saline and that the 10 mg/kg dose elicited significantly more licking, $t(27)=3.65$, and less sniffing, $t(27)=2.72$, than did saline. Since gnawing was the only response to occur infrequently in the absence of apomorphine and to occur at appreciably high levels in the presence of 1 and 10 mg/kg doses of apomorphine, all subsequent comparisons are restricted to the use of gnawing as a dependent variable.

Figure 2 shows the mean percent of gnawing as a function of 10 min time periods for each dose of apomorphine. As the dose of apomorphine increased the amount and duration of gnawing increased. The lowest apomorphine dose to elicit gnawing was 0.1 mg/kg, but the effect was transient, lasting 10–20 min. At the two highest doses, gnawing was robust and persistent. During the first half of the observation period, apomorphine doses of 1 and 10 mg/kg elicited equivalent amounts of gnawing, but during the last half of the period, gnawing elicited by the 1 mg/kg dose decreased at a faster rate than did that elicited by the 10 mg/kg dose. A 5×6 (Dose × Time) ANOVA indicated that the effects of dose, $F(4,162)=48.59$, time, $F(5,162)=7.35$, and the interaction between dose and time, $F(20,162)=2.04$, were significant.

Figure 3 (top) shows the mean percent of gnawing as a function of dose of apomorphine for animals receiving either morphine or saline prior to the injection of apomorphine in Experiment 2. As in the first experiment, apomorphine doses less than 1 mg/kg induced little or no gnawing, whereas the 1 mg/kg dose induced considerable gnawing. In addition, a 15 mg/kg dose of morphine itself induced gnawing, as can be seen by comparing morphine groups with saline controls. However, there was no evidence of morphine potentiation of apomorphine-induced gnawing at any dose of apomorphine. That is, morphine did not increase the amount of gnawing elicited by any dose of apomorphine beyond that elicited by morphine itself. A 4×2 (Apomorphine Dose × Morphine Dose) ANOVA indicated that the effect of apomorphine dose, $F(3,24)=20.33$, and the effect of morphine dose, $F(1,24)=6.11$, were significant, but that the interaction was not, $F(3,24)<1$. Subsequent t-tests indicated that, as in the first experiment, the 1 mg/kg dose of apomorphine elicited significantly more gnawing than did saline, $t(24)=6.33$.

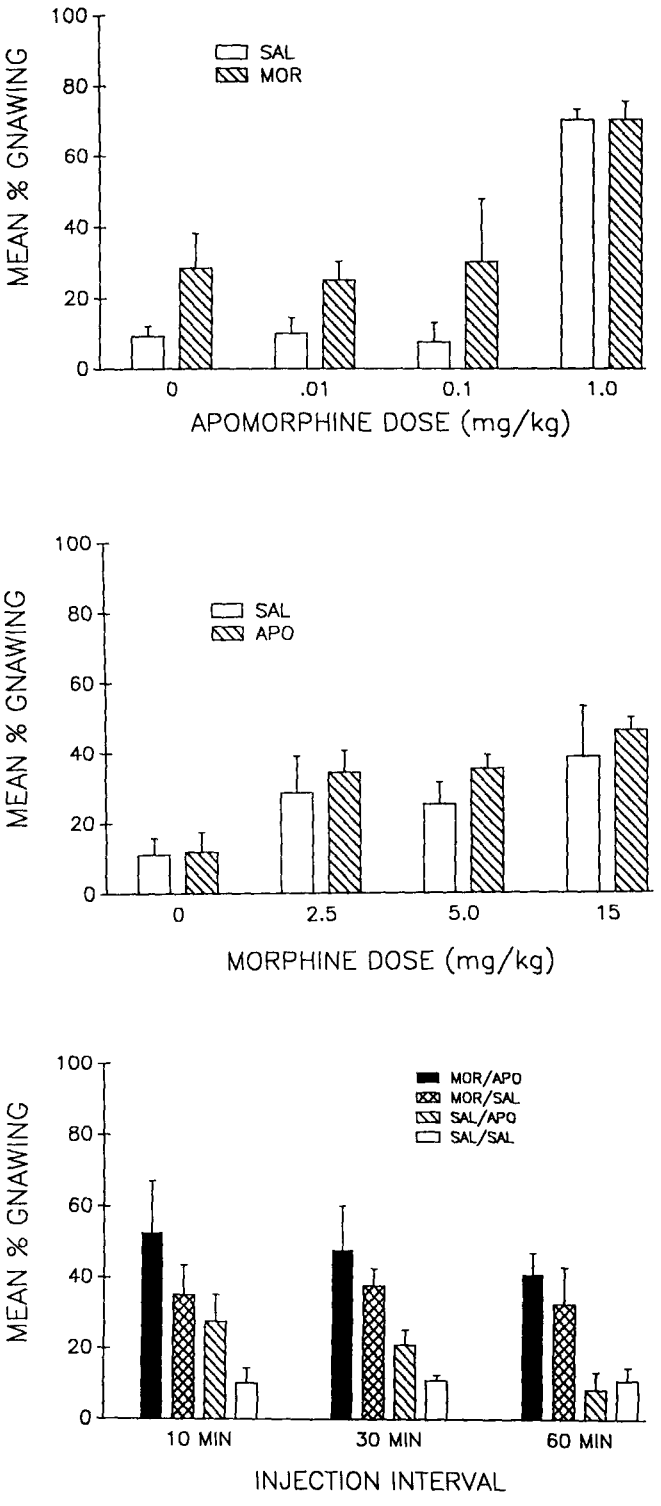

FIG. 3. (Top) Mean (+S.E.M.) percent of gnawing as a function of dose of apomorphine for groups (n=4) receiving either 15 mg/kg morphine (MOR) or saline (SAL) in Experiment 2. (Middle) Mean (+S.E.M.) percent of gnawing as a function of dose of morphine for groups (n=4) receiving either saline (SAL) or 0.1 mg/kg apomorphine (APO) in Experiment 3. (Bottom) Mean (+S.E.M.) percent of gnawing as a function of the interval between injections for Groups MOR/APO (n=4), MOR/SAL (n=4), SAL/APO (n=4), and SAL/SAL (n=4) in Experiment 4.

Figure 3 (middle) shows the mean percent of gnawing as a function of dose of morphine for animals receiving saline or apomorphine following the morphine injection in Experiment 3. Morphine-induced gnawing was clearly dose-related, but no dose of morphine potentiated the ineffective, low dose of apomorphine. A 4×2 (Morphine Dose × Apomorphine Dose) ANOVA indicated that the effect of morphine dose was significant, $F(3,24)=5.43$, but that neither the effect of apomorphine dose nor the interaction was significant. Subsequent t-tests indicated that the 2.5, 5 and 15 mg/kg doses of morphine elicited significantly more gnawing than did saline, $t(24)=2.58$, $t(24)=2.42$, $t(24)=3.97$, respectively.

Figure 3 (bottom) shows the mean percent of gnawing as a function of the time interval between the injections for the four treatment groups in Experiment 4. As in Experiments 2 and 3, morphine (15 mg/kg) elicited more gnawing than did saline, and a 0.1 mg/kg dose of apomorphine failed to induce reliably more gnawing than saline. Moreover, there was no morphine potentiation of apomorphine-induced gnawing at any of the time intervals tested. A 2×2×3 (Morphine Dose × Apomorphine Dose × Time) ANOVA indicated that the effect of morphine dose was significant, $F(1,12)=17.08$. None of the other main effects or interactions, however, was significant.

Figure 4 (top) shows the mean percent of gnawing as a function of dose of morphine for groups receiving naloxone or saline in Experiment 5. It is evident that morphine elicited gnawing in a dose-related manner and that naloxone blocked the effects of morphine. These conclusions are corroborated by a 2×4 (Naloxone Dose × Morphine Dose) ANOVA which indicated that the effects of morphine dose, $F(3,24)=4.46$, and naloxone dose, $F(1,24)=5.71$, were significant, but that the interaction was not. In addition, t-tests indicated that the 15 mg/kg, $t(24)=2.41$, and the 30 mg/kg, $t(24)=3.56$, doses of morphine elicited more gnawing than did saline and that, at the 15 mg/kg dose of morphine, naloxone blocked morphine-induced gnawing, $t(24)=2.62$, but that at the 30 mg/kg dose of morphine, naloxone antagonism was incomplete. That is, at the 30 mg/kg dose of morphine, the difference in gnawing between animals given saline and those given naloxone was not significant, $t(24)=1.77$.

Figure 4 (middle) shows the mean percent of gnawing as a function of the dose of apomorphine for groups receiving either saline or naloxone prior to apomorphine in Experiment 6. It appears that naloxone had only a small effect on apomorphine-induced gnawing. A 2×2 (Naloxone Dose × Apomorphine Dose) ANOVA indicated that the effect of apomorphine dose was significant, $F(1,28)=91.83$, but that neither the effect of naloxone dose nor the interaction was significant.

Figure 4 (bottom) shows the mean percent of gnawing as a function of dose of naloxone for groups receiving saline or apomorphine in Experiment 7. It is clear that apomorphine-induced gnawing was not antagonized by any dose of naloxone. A 2×5 (Apomorphine Dose × Naloxone Dose) ANOVA indicated that the effect of apomorphine dose was significant, $F(1,14)=69.89$, but that neither the effect of naloxone nor the interaction was significant.

Figure 5 shows the mean percent of gnawing as a function of either morphine, apomorphine or saline for animals receiving water vehicle or haloperidol in Experiment 8. Haloperidol selectively blocked apomorphine-induced gnawing without blocking morphine-induced gnawing. A 3×2 (Drug × Haloperidol Dose) ANOVA indicated that the effect of

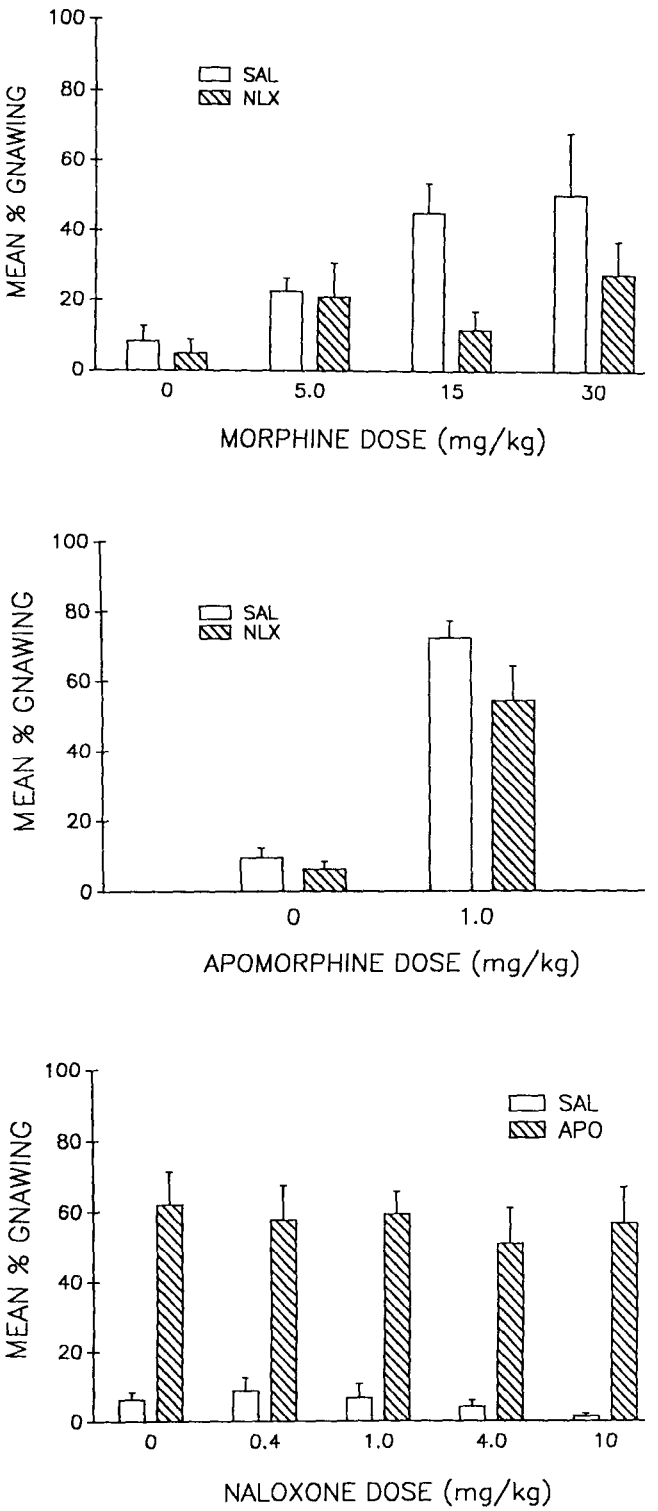

FIG. 4. (Top) Mean (+S.E.M.) percent of gnawing as a function of morphine dose for groups (n=4) receiving 0.4 mg/kg naloxone (NLX) or saline (SAL) in Experiment 5. (Middle) Mean (+S.E.M.) percent of gnawing as a function of apomorphine dose for groups (n=8) receiving either saline (SAL) or 0.4 mg/kg naloxone (NLX) in Experiment 6. (Bottom) Mean (+S.E.M.) percent of gnawing as a function of naloxone dose for groups (n=8) receiving saline (SAL) or 1 mg/kg apomorphine (APO) in Experiment 7.

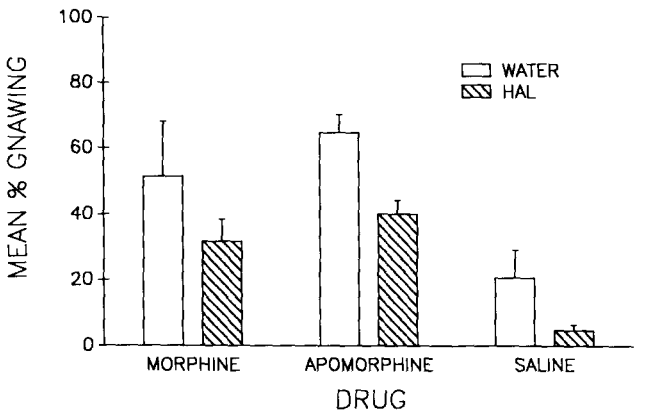

FIG. 5. Mean (+S.E.M.) percent of gnawing as a function of drug (30 mg/kg morphine, 1 mg/kg apomorphine or saline) for groups (n=5 or 6) receiving water or haloperidol (HAL) in Experiment 8.

drug (morphine, apomorphine or saline) was significant, $F(2,26)=12.17$, and that the effect of haloperidol dose, $F(1,26)=8.78$, was significant, but that the interaction was not. Subsequent t-tests indicated that morphine, $t(26)=3.44$, and apomorphine, $t(26)=4.74$, each induced more gnawing than did saline and that haloperidol decreased apomorphine-induced gnawing, but not morphine-induced gnawing. That is, haloperidol plus apomorphine elicited significantly less gnawing than did water plus apomorphine, $t(26)=2.13$, whereas haloperidol plus morphine did not produce significantly less gnawing than water plus morphine, $t(26)=1.70$. Finally, there was no significant effect of haloperidol among saline controls, $t(26)=1.38$.

DISCUSSION

It has been known for some time that apomorphine and morphine produce stereotyped behavior in rats, guinea pigs and mice (1–8, 10–14, 17). The present study extends these effects to the hamster. Moreover, the present results indicate that the effects of morphine and apomorphine on stereotyped gnawing are selective. That is, morphine-induced gnawing was effectively antagonized by a 0.4 mg/kg dose of the opiate antagonist naloxone, whereas neither that dose nor doses of 1, 4 or 10 mg/kg antagonized apomorphine-induced gnawing. Thus, the present results are consistent with those studies in the rat (1,7) showing no effect of naloxone on apomorphine-induced stereotypy. Conversely, apomorphine-induced gnawing but not morphine-induced gnawing was antagonized by a low dose of the dopaminergic antagonist, haloperidol. It might be hypothesized that a higher dose of haloperidol would have antagonized morphine-induced gnawing. That hypothesis, however, is difficult to evaluate since, at higher doses, haloperidol has significant sedating effects in the hamster. Thus, it would appear that morphine's effects on gnawing are mediated by an opioidergic system, whereas those of apomorphine are mediated by a dopaminergic system. Other investigators have identified this latter system in the rat as the nigrostriatal dopamine pathway (14).

Furthermore, despite testing a variety of doses of morphine and apomorphine, we could find no evidence that morphine potentiates apomorphine-induced gnawing in the hamster. In Experiment 2, a 15 mg/kg dose of morphine failed to increase the amount of gnawing elicited by either 0.01, 0.1 or 1.0 mg/kg doses of apomorphine. In Experiment 3, none of the three doses of morphine (2.5, 5.0 and 15 mg/kg) potentiated gnawing induced by a 0.1 mg dose of apomorphine. In Experiment 4, morphine administered 10, 30 or 60 min prior to apomorphine failed to potentiate apomorphine-induced gnawing. In each of these experiments, however, morphine itself elicited gnawing. It is unlikely that higher doses of morphine, which produce sedation in the hamster, would have potentiated the effects of apomorphine. Although other investigators have reported morphine potentiation of apomorphine stereotypy (3–5, 10–12, 14), many have failed to control for morphine-induced stereotypy [e.g., (10, 12, 14)]. Nevertheless, in one well-controlled study of the rat (5), morphine potentiated apomorphine stereotypy independently of its own effect on stereotyped behavior. In the hamster, however, morphine and apomorphine appear to act independently and noninteractively to control stereotyped behavior.

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