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Modulation of the Stimulus Effects of Morphine by *d*-Amphetamine

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GAIARDI, M., M. BARTOLETTI, C. GUBELLINI, A. BACCHI AND M. BABBINI. *Modulation of the stimulus effects of morphine by* d-*amphetamine.* PHARMACOL BIOCHEM BEHAV **59**(1)249–253, 1998.—The stimulus effects of morphine and *d*-amphetamine coadministration were studied in rats. Place conditioning, drug discrimination, and taste conditioning were employed to assess the rewarding, discriminative, and aversive stimulus properties of both drugs. *d*-Amphetamine increased the rewarding and morphine-like discriminative stimulus effects of 1.25 mg/kg morphine. *d*-Amphetamine did not, however, change the aversive effects of 1.25 mg/kg morphine, or any effect of higher (5–20 mg/kg) morphine doses. Because the rewarding/discriminative properties and the aversive properties of a drug are considered the main attributes that regulate (facilitate and weaken, respectively) drug-seeking behavior, the present data are in keeping with clinical reports indicating that amphetamines are sometimes used by opiate abusers in an attempt to increase the effect obtained from poor-quality heroin. © 1998 Elsevier Science Inc.

Morphine *d*-Amphetamine Rat Aversive properties Discriminative properties Rewarding properties

IT has been reported that receptors located within mesolimbic structures mediate, at least in part, the discriminative stimulus effects of morphine (21) and *d*-amphetamine (15). On the other hand, data implicating dopamine and opioid systems in *d*-amphetamine and morphine reward include critical elements in both the nucleus accumbens and ventral tegmental areas (12). Finally, a strong functional relationship or perhaps a commonality has been suggested between neurochemical systems mediating the rewarding and the aversive properties of drugs that are self-administered by animals (10). On the basis of such considerations additive effects should be expected not only for the rewarding/discriminative properties, as reported by some authors (4,14), but also for the aversive properties of morphine and *d*-amphetamine. The present study was performed to test this hypothesis in rats.

Place conditioning, drug stimulus discrimination, and taste conditioning were employed to assess, respectively, the rewarding (Experiment 1), discriminative (Experiment 2), and aversive (Experiment 3) effects of combinations of the two drugs.

EXPERIMENT 1

Method

Subjects. Male Sprague–Dawley rats (approximately 290–350 g at the beginning of the experiment) were housed in groups of four under standard laboratory conditions (lights on 0700–1900 h, temperature 22 ± 1°C). Food and water access was unlimited. All subjects were handled daily for 1 week before initiation of the experiment.

Drugs. Morphine hydrochloride (SALARS, Italy) and *d*-amphetamine sulphate (Recordati, Italy) were dissolved in saline. Doses are expressed as the salt. All treatments were administered IP (2 cc/kg).

Apparatus. The testing apparatus consisted of two rectangular interconnected chambers, enclosed in a dimly lit, sound insulated, and ventilated shell. One compartment had white walls, a 0.8-cm wire mesh floor and fir sawdust under the floor. The other compartment had black walls, a 1.2- cm wire mesh floor, and poplar sawdust under the floor. A 15 × 6-cm

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aperture with a sliding door connected the sides. Three photocells measured the time spent by the rat in each compartment.

Procedure. Rats (n = 135) were randomly assigned to 16 groups (n = 7–8 per group; control group: n = 16) and received three pairings of drug (morphine: 0, 1.25, 5, 20 mg/kg plus *d*-amphetamine: 0, 0.5, 1, 2 mg/kg) with one set of cues and three pairings of saline (two injections) with the other set of cues in different days (days 1–6). During a typical session, the rat was given an injection of morphine, followed 5 min later by an injection of *d*-amphetamine, and then was placed for 30 min into its training compartment. Assignment to particular cues and order of treatments was always balanced for the animals of a particular group (i.e., in each group for half the animals the drug-paired side was the right one, for the other half the left one; furthermore, the first conditioning session was a drug session for half the animals, and a saline session for the other half). Testing was carried out on day 7. On this occasion no injections were given; animals were placed in the apparatus and allowed to explore both compartments for 30 min; initial placement was counterbalanced within each group.

Statistical analysis. The time (s) spent on the drug-paired side was expressed as difference from the total test time (which was slightly different from session to session due to a rather inaccurate timer). Thus, times are presented as negative values and higher (less negative) values indicate greater preference for the drug-associated place. Morphine and *d*-amphetamine dose–effect curves were fitted using the method of the orthogonal polynomials of least squares. Further comparisons were performed using the F-test.

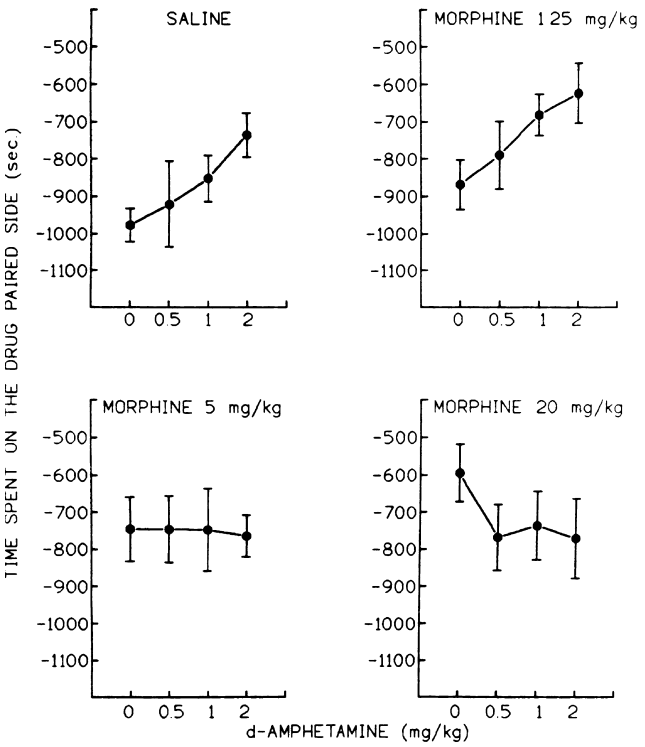

FIG. 1. Time spent on the drug-paired side (expressed as difference from total test time) after place conditioning with morphine in combination with various doses of *d*-amphetamine. Each point represents the mean ± SEM.

Results

The data are summarized in Fig. 1. Morphine alone caused a dose-related preference for the drug-associated place, $F(1, 35) = 19.28$, $p < 0.01$. Furthermore, the analysis revealed a significant linear relation between *d*-amphetamine dose (including 0) and time spent on the drug paired side in animals pretreated with saline, $F(1, 36) = 7.18$, $p = 0.01$, or 1.25 mg/kg morphine, $F(1, 28) = 6.44$, $p < 0.05$. In both cases a significant *d*-amphetamine effect was observed at a dose of 2 mg/kg [saline: $F(1, 36) = 6.87$, $p = 0.01$; 1.25 mg/kg morphine: $F(1, 28) = 5.43$, $p < 0.05$. On the other hand, no dose-related effect of amphetamine was observed in animals pretreated with 5 ($F < 1$) or 20, $F(1, 27) = 1.39$, p = NS, mg/kg morphine; furthermore, the overall morphine plus *d*-amphetamine effect was not significant against morphine alone [5 mg/kg morphine: $F < 1$; 20 mg/kg morphine: $F(1, 27) = 2.16$, p = NS.

EXPERIMENT 2

Method

Subjects. See Experiment 1, except that animals were housed three to a cage and food was restricted to 54 g per cage given at 1930 h.

Drugs. See Experiment 1.

Apparatus. Operant chambers were equipped with a food tray (for 70 mg food pellets) and two levers (left and right). Each box was enclosed in a dimly lit, sound-insulated, and ventilated compartment.

Procedure. Rats were trained to discriminate morphine (10 mg/kg) from saline in a two-lever food-reinforced operant task (tandem VI 60 FR10). The subjects were placed in the operant chambers 30 min after the treatment and were allowed to respond for 30 min. The number of responses the animal made on either lever before obtaining the first reinforcement (FRF) was recorded following each trial. Test sessions began when a subject reached the training criterion consisting of FRF $\leqslant$ 12 on at least eight out of nine consecutive daily training sessions. The training procedure was continued during the test period and testing was postponed if the FRF exceeded 15 on either of the two most recent training days. On test days rats were given an injection of *d*-amphetamine (0, 0.25, 0.5 mg/kg) followed 5 min later by an injection of morphine (0, 1.25, 5, 10 mg/kg). On these occasions it was noted on which lever the rat totalized 10 responses first (selected lever); then the rat was given its first food pellet and was reinforced throughout the trial (tandem VI 60 FR10) upon pressing the selected lever.

Statistical analysis. Percents of rats selecting the morphine lever after morphine, *d*-amphetamine, or both were tested for linear trend. Further comparisons were performed using χ^2 test.

Results

The data are summarized in Fig. 2. Morphine alone caused a dose-related increase in the percentage of rats selecting the drug appropriate lever ($\chi^2_1 = 34.15$, $p < 0.01$). No significant linear relation between *d*-amphetamine dose (including 0) and drug lever selection was observed, except in animals pretreated with 1.25 mg/kg morphine ($\chi^2_1 = 4.80$, $p < 0.05$); in this case a significant *d*-amphetamine effect was observed at a dose of 0.5 mg/kg ($\chi^2_1 = 4.84$, $p < 0.05$).

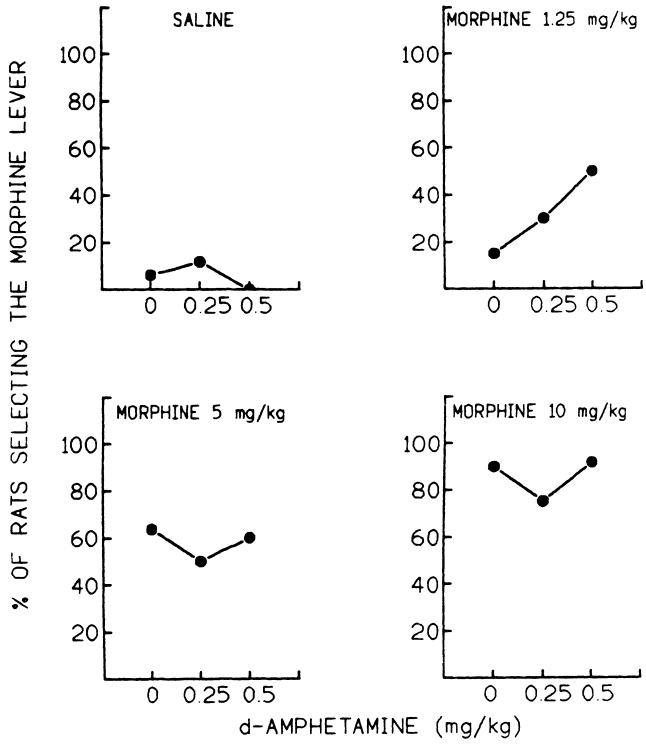

FIG. 2. Percent drug lever selection in rats trained to discriminate 10 mg/kg morphine from saline and tested with morphine in the presence of *d*-amphetamine.

EXPERIMENT 3

Method

Subjects. See Experiment 1, except that animals had water available only for 90 min a day during the last 2 days before the initiation of the experiment and were water deprived for about 23 h before each trial.

Drugs. See Experiment 1.

Apparatus. The apparatus consisted of standard operant chambers equipped with a grid floor and one or two bottles located on opposite walls. Both grid floor and bottles were connected to a drinkometer circuit. As suggested by Vogel et al. (26), to provide a reliable measure of consummatory behavior, the circuit produced seven pulses for second whenever the rat was in contact with the bottle; one pulse was counted as equivalent to one lick.

Each box was enclosed in a dimly lit, sound-insulated and ventilated compartment.

Procedure. Rats were randomly assigned to twelve groups (n = 8–9 per group) and received three pairings of drug (morphine: 0, 1.25, 5, 20 mg/kg plus *d*-amphetamine: 0, 0.25, 0.5 mg/kg) with 0.1% saccharin and three pairings of saline (two injections) with water in different days (days 1–2, 4–7). During a typical session the rat was placed for 20 min in the apparatus; immediately following the conditioning session, the rat was given an injection of morphine, followed 5 min later by an injection of *d*-amphetamine. The bottle placement (right, left) and the order of conditioning sessions were counterbalanced within each group. Testing was carried out after two (day 3) and six (day 8) conditioning sessions. On these occasions animals were placed in the apparatus and allowed to drink both saccharin and water for 20 min.

Statistical analysis. Saccharin licks were expressed as percent of the corresponding total licks and logit transformed to correct heterogeneity of variance. Because the "test" effect was never significant, the data were collapsed for this main effect. Morphine and *d*-amphetamine dose–effect curves were fitted using the method of the orthogonal polynomials of least squares. Further comparisons were performed using *F*-test.

Results

The data are summarized in Fig. 3. Morphine alone caused a dose-related aversion for saccharin, $F(1, 62) = 36.05$, $p < 0.01$. Furthermore, the analysis revealed a significant linear relation between *d*-amphetamine dose (including 0) and saccharin licks only in animals pretreated with saline, $F(1, 46) = 12.43$, $p < 0.01$. A significant *d*-amphetamine effect was observed at both doses [0.25 mg/kg: $F(1, 46) = 4.88$, $p < 0.05$; 0.5 mg/kg: $F(1, 46) = 12.43$, $p < 0.01$]. On the other hand, no dose-related aversion for saccharin was observed in animals pretreated with 1.25, $F(1, 48) = 2.95$, p = NS, 5, $F(1, 44) = 1.89$, p = NS, or 20 ($F < 1$) mg/kg morphine; furthermore, the overall morphine plus *d*-amphetamine effect was not significant against morphine alone (1.25 mg/kg morphine: $F = 1$; 5 mg/kg morphine: $F < 1$; 20 mg/kg morphine: $F < 1$).

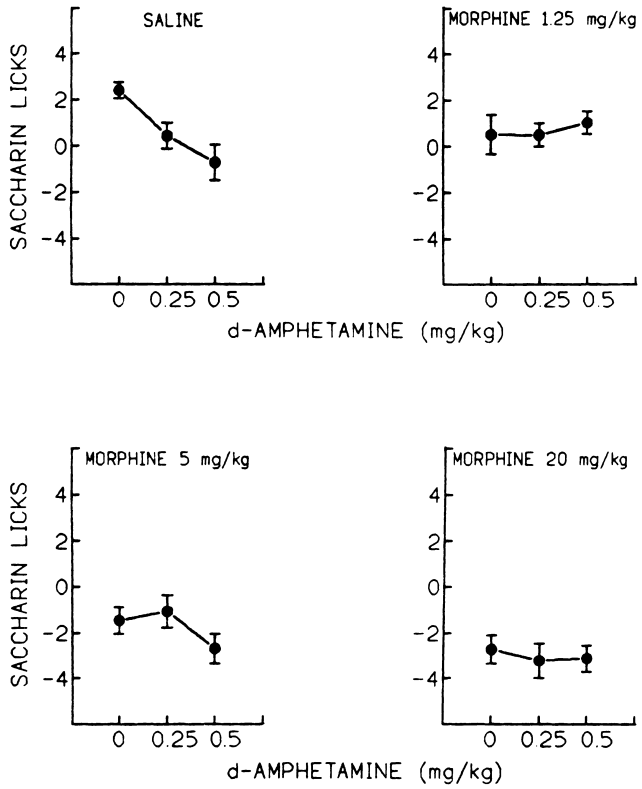

FIG. 3. Saccharin licks (expressed as percent of the corresponding total licks and logit transformed) after taste conditioning with morphine in combination with *d*-amphetamine. Because the "test" effect was not significant (see text), the data are collapsed for this main effect. Each point represents the mean ± SEM.

GENERAL DISCUSSION

Morphine and *d*-amphetamine caused, as expected (3,10), place preference and taste aversion. The preference shift induced by a low morphine dose (1.25 mg/kg) was increased in combination with *d*-amphetamine. In this regard, it is worth noting that the place preference induced by 1.6 mg/kg morphine was enhanced in combination with methamphetamine (14). Furthermore, pronounced decreases in the threshold for rewarding intracranial electrical stimulation were seen when an ineffective or minimally effective dose of morphine was administered in combination with various doses of *d*-amphetamine (9). The preference shift induced by higher morphine doses (5 and 20 mg/kg) and the morphine-induced taste aversion were unaffected by the concomitant administration of *d*-amphetamine. A similar result was found by Suzuki et al. (23), who reported that the combination of morphine and methamphetamine did not enhance taste aversion. These findings could reflect a phenomenon such as overshadowing; thus, the presence of morphine could have interfered with the acquisition of control by *d*-amphetamine, a possibly less salient stimulus at the doses used. Rats trained to discriminate 10 mg/kg morphine from saline did not show crossgeneralization to *d*-amphetamine. In this regard it is worth noting that animals trained to a low, but not to a moderate or a high morphine dose, generalized to the *d*-amphetamine stimulus (4). The discriminative stimulus properties of low (1.25 mg/kg) and high (5 and 10 mg/kg) morphine doses were potentiated and unaffected respectively by *d*-amphetamine. A morphine potentiation has been previously reported by Gauvin and Young (4) in pigeons trained to discriminate 10 mg/kg morphine from saline and receiving 1 (but not 1.8 or 3.2) mg/kg *d*-amphetamine. On the other hand, in pigeons trained to a three-choice discrimination including two morphine doses the discriminative stimulus properties of the high training dose were not altered by any *d*-amphetamine challenge dose (5); furthermore, in rats trained to discriminate 5.6 (but not 3.2) mg/kg morphine, *d*-amphetamine (0.1 to 1 mg/kg) did not modify the stimulus effect of the training dose (27). Thus, in animals trained to a high morphine dose, morphine–amphetamine combination generally produce potentiation or indifference, depending on morphine and, to a much a lesser extent, amphetamine test doses.

In summary, *d*-amphetamine, at the doses used, increased the rewarding and the discriminative, but not the aversive stimulus properties of 1.25 mg/kg morphine. It has been reported that receptors located within mesolimbic structures mediate, at least in part, the discriminative stimulus effects of morphine and *d*-amphetamine (15,21). Furthermore mesoaccumbens dopamine–opiate interactions are possibly critical also in mediating the rewarding effects of morphine and *d*-amphetamine (8,12). In fact, D_1 antagonists (1,19) or bilateral 6-hydroxydopamine lesions of the nucleus accumbens (19) abolished morphine-induced place preference. Furthermore narcotic antagonists blocked amphetamine (25) and methamphetamine (24) place preference. Thus, our data, indicating that discriminative/rewarding effects are potentiated by the concomitant administration of amphetamine and morphine, are in line with available evidences indicating a strong functional relationship between the underlying neurochemical actions of the two drugs. A prominent role of dopamine in mediating the aversive properties of amphetamine has been demonstrated by attenuating the effect using pimozide (7) and 6-hydroxydopamine lesions of the central dopamine neurons (18). Furthermore, nucleus accumbens D_1 receptors have been implicated in the mediation of the aversive effects of opioids (19). Thus, additive or supradditive effects should have been obtained for morphine and amphetamine in taste conditioning, too. However, it has been suggested that a peripheral as well as a central component may contribute to the aversive effects of sistemically administered opioids (20). Moreover, amphetamine-induced taste aversion possibly has both a central dopaminergic component and a nondopaminergic peripheral component (17). Finally, for both drugs the peripheral component seems to be mainly relevant at low doses (2,16). Thus, the fact that the adversive effects are not potentiated by the concomitant administration of morphine and amphetamine could be related to the peripheral rather than central component of their action.

The opiates and the central nervous system stimulants are two classes of drugs that are widely abused. Although a significant degree of euphoria is associated with the administration of these substances on an individual basis, polidrug abuse between these drug classes has also been observed (13). Furthermore, a study conducted in human subjects suggested that the combination of morphine and *d*-amphetamine causes additive euphoria and a lessening of side effects (11). According to Stolerman (22), the rewarding/discriminative effects of drugs facilitate drug seeking, while the aversive effects weaken the behavior; thus, they can be considered as a model of euphoria and side effects, respectively. Within this framework our results are, at least partly, consistent with clinical data. The effects seem to be limited to low morphine doses. On the other hand, it has been reported that amphetamines are used by opiate abusers in an attempt to increase the effect obtained from methadone or poor quality heroin (6).

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